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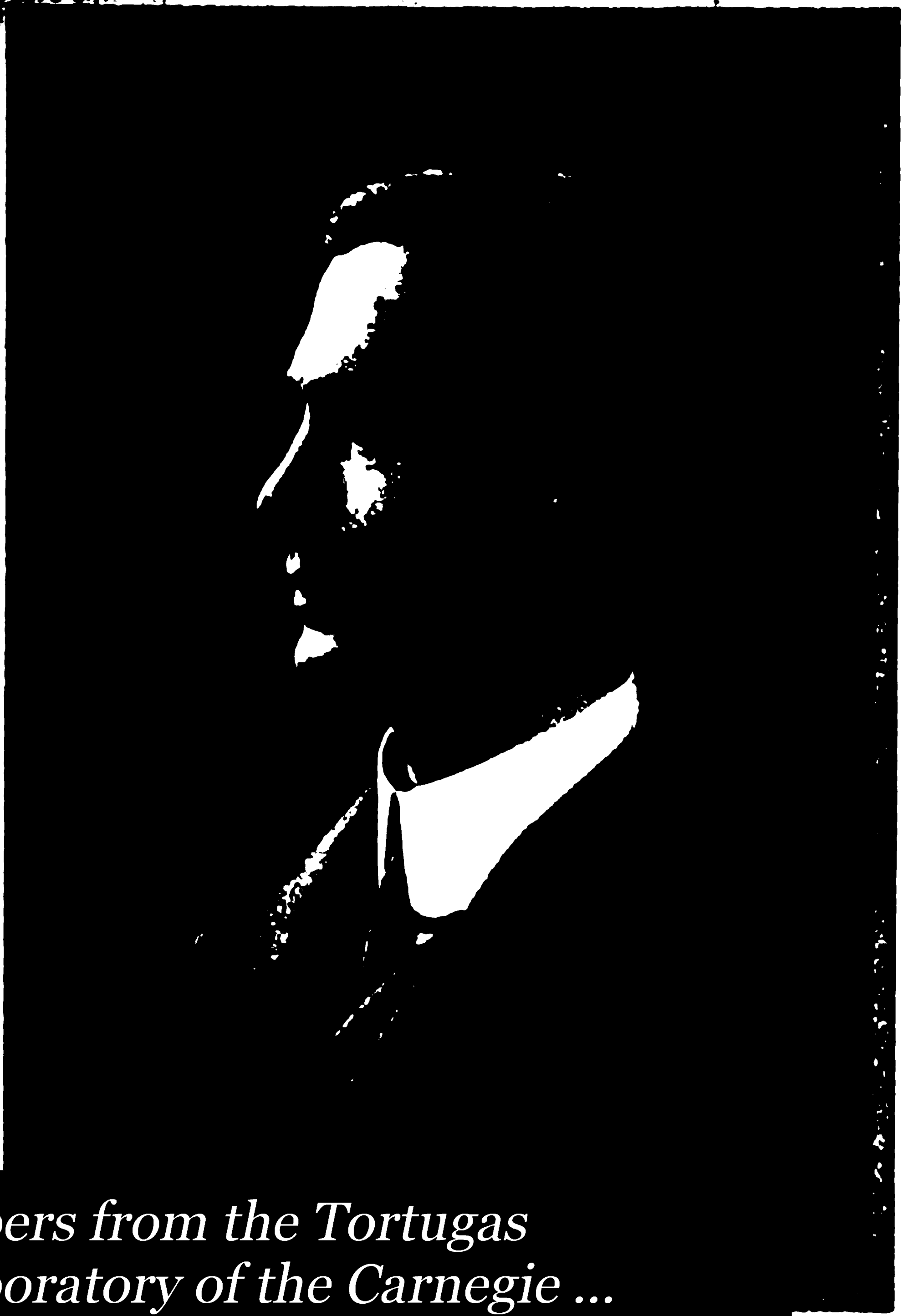

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DEPARTMENT OF MARINE BIOLOGY
OF
CARNEGIE INSTITUTION OF WASHINGTON

ALFRED G. MAYER, DIRECTOR

PAPERS FROM THE TORTUGAS LABORATORY
OF THE
CARNEGIE INSTITUTION OF WASHINGTON

VOLUME V

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1914

CARNEGIE INSTITUTION OF WASHINGTON

PUBLICATION NO. 182

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PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

189063
OCT -6 1914
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CONTENTS.

Page.
I. In Memoriam: George Harold Drew. By **Alfred G. Mayer**....1 plate 1–6
II. On the Precipitation of Calcium Carbonate in the Sea by Marine Bacteria, and on the Action of Denitrifying Bacteria in Tropical and Temperate Seas. By G. H. **Drew**................2 maps, 4 figures 7–45
III. Preliminary Remarks on the Geology of the Bahamas, with Special Reference to the Origin of the Bahaman and Floridian Oolites. By T. **Wayland Vaughan**... 47–54
IV. Building of the Marquesas and Tortugas Atolls and a Sketch of the Geologic History of the Florida Reef Tract. By T. **Wayland Vaughan**.. 55–67
V. Some Chemical Characteristics of Sea-water at Tortugas, Florida. By R. B. **Dole**.. 69–78
VI. Observations upon the Growth-rate and Œcology of Gorgonians. By L. R. **Cary**..2 plates 79–90
VII. Growth-Changes in Brittle-Stars. By H. L. **Clark**...........3 plates 91–126
VIII. The Early Influence of the Spermatozoan upon the Characters of Echinoid Larvæ. By D. H. **Tennent**........................11 figures 127–138
IX. Studies of Jamaica Echini. By **Robert T. Jackson**..........21 figures 139–162
X. The Spermatogenesis of the Mongoose; and a Further Comparative Study of Mammalian Spermatogenesis, with Special Reference to Sex Chromosomes. By H. E. **Jordan**.............1 plate, 9 figures 163–180
XI. Bryozoa of the Tortugas Islands, Florida. By R. C. **Osburn**....23 figures 181–222

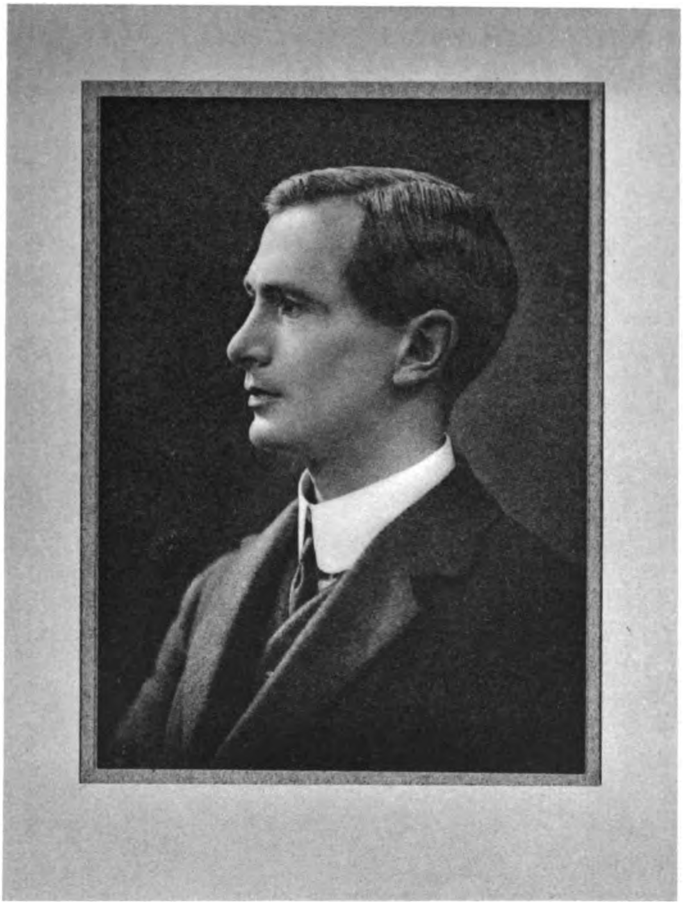
I.

IN MEMORIAM.

GEORGE HAROLD DREW,

1881-1913.

One plate.



GEORGE HAROLD DREW

GEORGE HAROLD DREW.

(1881–1913.)

George Harold Drew, only son of George Drew, of Devonshire, England, was born on October 23, 1881, and died suddenly on January 29, 1913.

From early boyhood chemistry and microscopy fascinated him, and while yet a child he studied in a disused stable on his father's place, which was made to serve as a rough laboratory.

Until the age of ten he was taught by his mother, and then he was sent to New College, Eastbourne, Sussex. Being deeply interested in the scientific side of medicine, he entered Cambridge University, where he became a scholar of Christ's College, and gained the distinctions of Exhibitioner, Foundation Scholar, and Prizeman, afterwards becoming Schreiner University Scholar, and a student at Exeter Hospital. During his five years at Cambridge he took the entire medical course, but afterwards decided to devote his life to research, inclination leading him to choose the scientific side of his profession rather than to engage in its active practice.

After leaving Cambridge, he served for a year as University Scholar of St. Mary's Hospital, London, and subsequently, after working for a short time at the Port Erin Marine Laboratory, he went to Plymouth and associated himself with the Marine Biological Laboratory, wherein most of his scientific work was accomplished. He was one of those chosen by Captain Scott to serve upon the scientific staff of the British Antarctic Expedition, but he declined the honor, preferring not to break the continuity of his studies at Plymouth.

From 1910 until 1912 he held a Beit Memorial Fellowship for Medical Research in cancer; in the summer of 1912 he was appointed John Lucas Walker research student in the University of Cambridge, and on January 1, 1913, he became a Research Associate of the Department of Marine Biology of the Carnegie Institution of Washington.

His connection with the Carnegie Institution of Washington began in 1911, when he accompanied the expedition to Jamaica, afterwards studying at the Tortugas, and upon a second visit to America in 1912 he spent a month at the temporary laboratory established at Golding Cay, Andros Island, Bahamas.

He was to have been a member of the projected expedition to Torres Straits, Australia, in 1913, and his preparations were being matured at the time of his sudden death.

The saddest losses that the world sustains are often those it heeds the least, and all who knew him realize that this young man, with his keen insight for research, his broad culture, and well-rounded special training, was destined to become one of England's great men of science—a position which his rare personal charm would not only have graced, but would have rendered him powerful for advancing the intellectual welfare of his country.

In 1910 he carried out some interesting observations upon the reproduction and early development of the kelp (*Laminaria*) of the coast of Devonshire, proving that there is an alternation of generations in the life cycle of this seaweed; for the free-swimming sexual cells fuse in pairs and then settle down upon the bottom and develop into a chain of cells, any single cells of which may develop into a *Laminaria* plant.

His published works cover a period of only four years, yet in this brief time he was the author or joint author of fifteen papers.

His earliest paper, in 1909, is upon parasitic and other diseases of fish, in which he describes various forms of tumors and cancers.

This paper was followed by one of an experimental nature written in association with de Morgan as its joint author, and it was shown that, in *Pecten*, injurious bodies implanted in the tissues soon become surrounded by an agglutinated layer of blood-corpuscles and these are replaced by a mass of fibrous tissue which encapsules the foreign body very much as such bodies are incased by mesenchyme in injured vertebrates.

In 1911 Drew announced that, as a result of injury, cysts lined by columnar ciliated epithelium could be formed from the fibroblasts in *Pecten*. He also found that the blood corpuscles of Lamellibranchs are capable of ingesting and destroying bacteria, and that if the animal be wounded so that blood escapes, some of the corpuscles adhere to the cut surfaces and then send out slender processes which fuse with those from other corpuscles, thus forming a net-work the fibers of which finally contract and close the wound.

He also found that the repeated application of a strong solution of iodine to a circumscribed area of the skin of *Fundulus* produces an inflammatory tumor having some relation to certain abnormal growths seen in nature in fishes.

While at Plymouth the subject of the cause and nature of tumors and cancers engaged his major interest, and he did much histological work upon the fate or growth of transplanted tissues. His last work was upon the culture of living tissues from the frog and the dogfish in plasma outside the body of the animal. In this study he achieved decided success, having with his usual ingenuity devised an improved technique which enabled him to grow tissues upon microscope slides free from bacterial contamination. The paper describing these results was completed only a few days before he died and is published in the Journal of Pathology and Bacteriology, Cambridge.

Some of his most interesting work, however, was done while associated with the Department of Marine Biology of the Carnegie Institution of Washington.

In 1910 Sanford, and also Vaughan, published the conclusion that a considerable portion of the calcareous muds in the bays and sounds of southern Florida were precipitated out of the sea-water in some unknown manner. It remained for Drew, in 1911, to discover that there is in the warm surface waters of the West Indian and Florida region, and especially in the limestone mud itself, a bacillus which deprives the sea-water of its nitrogen, thus causing the calcium to combine with the dissolved carbon dioxide and to form the finely-divided limestone mud so characteristic of coral-reef regions. Drew isolated this bacillus and found that it became inactive in even moderately cold water, and thus it functions only in warm or tropical seas, thriving best at depths of less than 100 fathoms. In the surface waters of the Bahamas and Florida it is the most abundant marine bacillus.

Drew hoped to continue these studies and to extend them to the Pacific, for this calcium precipitation is an important factor, and has resulted in the formation of vast beds of limestone apparently far greater in bulk than that formed by corals.

The complex problem of the chemical balance of the constituents of sea-water and their solvent powers under various conditions was being enthusiastically considered by Drew, and had he lived it was the hope of this Laboratory that he might have had exceptional opportunities to continue these studies—for truly he was the exceptionally brilliant man, for the coming of whose like again the world must wait, we fear, for many years, for there are but few in each generation who are gifted with his rare genius for research.

ALFRED GOLDSBOROUGH MAYER.

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II.

ON THE PRECIPITATION OF CALCIUM CARBONATE IN THE SEA BY MARINE BACTERIA, AND ON THE ACTION OF DENITRIFYING BACTERIA IN TROPICAL AND TEMPERATE SEAS.

BY G. HAROLD DREW.

Two maps and four figures.

TABLE OF CONTENTS.

Page.
Introduction 9
General considerations and previous work 10
Description of apparatus 13
Culture media and methods 19
The investigation of samples of sea-water taken off Port Royal, Jamaica 21
The investigation of samples of sea-water taken around the Dry Tortugas 24
The investigation of samples taken from a point 70 miles west of Ushant Island, France 27
The investigation of samples from the Marquesas Keys, Florida, and experimental precipitation of calcium carbonate by bacterial agency 29
Some considerations on the physiography of the Tongue of the Ocean and Andros Island 31
Bacterial investigations in the deep water of the Tongue of the Ocean 34
Hydrographic observations in the Tongue of the Ocean 37
Bacterial investigations of the chalky mud flats which are being deposited to the west of Andros Island 41
Conclusion 44

ON THE PRECIPITATION OF CALCIUM CARBONATE IN THE SEA BY MARINE BACTERIA, AND ON THE ACTION OF DENITRIFYING BACTERIA IN TROPICAL AND TEMPERATE SEAS.

BY G. HAROLD DREW.

INTRODUCTION.

The investigations described in this paper were made in the summers of 1911 and 1912 under the auspices of the Carnegie Institution of Washington. The original intention was to study the action of marine denitrifying bacteria in tropical seas. The discovery, during the course of the experiments, that these denitrifying bacteria also possess the power of precipitating calcium carbonate from soluble calcium salts present in sea-water has perhaps, by its geological significance, somewhat overshadowed the interest of the primary object of the work. The main contentions raised in this paper are:

(1) That in the seas of the American tropics bacteria exist which are actively precipitating calcium carbonate from the calcium salts present in solution in sea-water. It is suggested that this bacterial action has been a very considerable factor in the formation of chalk and many other varieties of sedimentary rock, chiefly or in part composed of calcium carbonate. It is also contended that the vast deposits of chalky mud now being formed to the west of the Bahamas and in the neighborhood of some of the Florida Keys are precipitated by bacterial agency and that a similar process plays an important part in the cementation of fragments of coral and other detritus into compact coralline rock.

(2) That the destruction of nitrates by bacterial action in the seas of the American tropics is far in excess of that occurring in temperate waters. Hence an explanation is afforded of the relative scarcity of plant life (and consequently of animal life) in tropical as compared to temperate seas, in accordance with the terms of Brandt's (2 and 3)[1] hypothesis.

Preliminary notes on this work have already been published in the Tortugas Laboratory Reports[2] for 1911 and 1912. The chronological sequence of the investigations will be followed in the account given here of the experimental work.

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[1] The figures in parentheses refer to the bibliography, p. 45.

[2] Carnegie Institution of Washington, Year Book No. 10, 1911, pp. 136–141; Year Book No. 11, 1912, pp. 136–144.

TABLE OF CONTENTS.

Introduction
General considerations and previous work
Description of apparatus
Culture media and methods
The investigation of samples of sea-water taken off Port Royal, Jamaica
The investigation of samples of sea-water taken around the Dry Tortugas
The investigation of samples taken from a point 70 miles west of Ushant Island, France
The investigation of samples from the Marquesas Keys, Florida, and experimental precipitation of calcium carbonate by bacterial agency
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GENERAL CONSIDERATIONS AND PREVIOUS WORK.

It is generally conceded that the plankton of tropical and subtropical seas is far less in quantity than that found in colder waters.[1] The zoo-plankton depends ultimately for its food on the phyto-plankton; hence any factor limiting the growth of the phyto-plankton which was capable of exercising its influence in tropical and not in temperate or arctic waters might offer an explanation of this phenomenon. It has been shown by various investigators that this factor is not temperature, light, or salinity, and it has been suggested that the explanation may lie in the relative deficiency, in tropical seas, of the nitrates or nitrogenous compounds so essential for all plant life. A matter of common observation in support of this view is the remarkable scarcity of algal growth in the shallow waters of tropical shores as compared with that in temperate regions, and the fact that in the tropics, wherever sewage or other nitrogenous waste is poured into the sea, a free growth of algæ is found.

There is at present no really reliable and accurate chemical method of estimating the combined nitrogen in sea-water, hence the above theory can not be directly put to the test. On the other hand, the existence of denitrifying bacteria in temperate waters has long been known, and it would seem a fair deduction that should this bacterial destruction of nitrates take place with greater intensity and completeness in tropical than temperate waters, an explanation of the relative scarcity of phyto-plankton in the former would be offered. This suggestion was first made by Brandt (3) in 1901, and is universally known as "Brandt's hypothesis." He enunciated it as follows:

> If the denitrifying bacteria of the sea, like the closely investigated denitrifying bacteria of the land, develop a strongly disturbing activity at higher temperatures, only a relatively small production (of phyto-plankton) would take place in the warm seas in spite of much more favorable conditions, according to the law of the minimum, owing to the great disturbance amongst the indispensable food substance; whilst, in the cold seas, more nitrogen compounds would be at the disposal of the producers owing to the retardation or suppression of the disturbing process. (From the published English translation.)

The presence of denitrifying bacteria has been demonstrated in Kiel Bay by Baur (1), along the Dutch coast by Gran (9), in the open waters of the North Sea and Baltic by Feitel (7) and Brandt (2), and in 1909 I identified several of the species described by Gran in samples of water obtained from the western part of the English Channel. All these denitrifying species have a higher temperature optimum than that of their natural environment and this is obviously a point strongly in favor of Brandt's hypothesis.

The chief difficulty in the way of putting the hypothesis directly to proof lies in the fact that at present no accurate method of determining the nitrate contents of sea-water exists, and hence it is impossible to correlate quantitative plankton observations with direct analyses of the amount of combined nitrogen present in sea-water in different localities. Much valuable work on this subject has been done by Raben (15), but he states that his error in control experiments averages over 30 per cent. An exhaustive study (as yet unpublished) of all the methods of estimating combined nitrogen in sea-water as given by various investigators has been made by Mr. D. J. Matthews, hydrographer to the Marine Biological Association of the United Kingdom and to the Fishery Branch of the Department of Agriculture, etc., for Ireland, and he has come to the conclusion that the limits of error in all these methods are so large as to make them unsuitable for investigations such as these. Since chemical methods are at present inadequate to give evidence on this hypothetical deficiency of nitrates in warmer seas, it seemed of interest to investigate the distribution and relative activity of denitrifying bacteria in tropical waters in comparison to those found in temperate seas, and it was with this primary object that the present work was undertaken.

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[1] For the most recent work, and full discussion of this subject, see The Depths of the Ocean, by Murray and Hjort, p. 366 et seq., 1912. London.

The previous researches most closely related to these investigations are those of Gran (9), who isolated a number of species of denitrifying bacteria from the inshore waters of the Dutch coast. He made use of solutions of nitrates, nitrites, or ammonium salts as the sole source of nitrogen in his culture media, which contained only a dilute solution of calcium malate as organic nutrient material for the bacteria. He classifies the bacteria into four groups, according to their reactions in pure cultures towards nitrates or nitrites.

1. Those which reduce nitrates and nitrites to free nitrogen without any ammonia formation.
2. Those which readily reduce nitrates to nitrites. The nitrite disappears slowly without perceptible formation of free nitrogen and some ammonia is formed.
3. Those which can not reduce nitrates to nitrites, but which are capable of slowly removing the nitrite without perceptible formation of free nitrogen. Though the nitrites are not reduced, yet they can serve as the sole source of nitrogen for the growth of the bacteria.
4. Those which can not reduce and are not capable of assimilating either nitrates or nitrites, but will flourish when ammonium salts are present.

In investigations on samples of water taken in the English Channel some 10 miles off Plymouth, I was able to recognize species belonging to the second group of Gran's classification, but could not detect the presence of species belonging to any of the other groups, and it would seem probable that these other groups are chiefly composed of littoral forms.

In fluid culture media inoculated with samples of sea-water and kept at a temperature of 28° C., Gran found that the formation of nitrite was detectible in from 1 to 2 days, and that eventually all the nitrate and nitrite was destroyed in the majority of cases, especially if the cultures were reinoculated at intervals. In my experiments I was able to obtain similar results in cultures kept at 30° C. after 8 days; in cultures kept at 15° C. the first formation of nitrite was detectible in from 5 to 6 days, but denitrification never proceeded beyond this stage.

Baur (1) showed that the optimum temperature for growth and denitrification of the species described by him lay between 20° and 25° C. when the bacteria were grown in fluid culture media containing peptone.

The most important work on the distribution of marine bacteria is that of Fischer (8) in 1886, 1889, and 1893, but he does not enter into the chemical activities of the species found, so that the observations do not throw much direct light on problems of the metabolism of the sea. The variations in the number of bacteria found in different surface samples from positions in mid-ocean are somewhat surprising and difficult to account for. Deeper samples were taken by means of a water-bottle made of brass, but in view of the now well-known bactericidal action of metals, and of copper in particular, I do not consider that any great value can be attached to these observations. With the exception of Fischer's work little seems to have been published on the general distribution of marine bacteria.

A point that has not yet been considered is the origin of the nitrate supply in the sea. Nitrates are absorbed by diatoms and the phyto-plankton in general, and are presumably built up into complex nitrogenous compounds within the plant. If these compounds, on the death of the plant, are broken up and the nitrogen again rendered available for use in the form of nitrates, a series of reactions must be gone through which may well be performed by bacterial agency, and this also applies to the waste nitrogenous products of animal metabolism. In addition, it has been shown that nitrates are actually decomposed by the denitrifying bacteria, which would thus tend to keep the nitrate concentration down to the level necessary for their own existence and would come into competition for this essential with other forms of plant life. If the bacteria are successful in decomposing nitrates to the extent of entirely removing the nitrogen from all chemical combination, as seems probable from the experiments in cultures, it follows that there must be some source of nitrates in order that the concentration in the sea may remain constant. The existence of nitrifying bacteria, which are capable of absorbing and combining with the free nitrogen of the air and eventually giving rise to nitrates, has been shown by Keding (10) and Keunter (11), but these have so far only been found on the bottom close to shore, or apparently living in symbiosis with algæ or plankton organisms. Similarly Thomsen (16) has demonstrated the presence on the bottom of inshore waters of bacteria which are capable of forming nitrites from ammonium salts, and others which can convert nitrites into nitrates. It would seem possible that similar bacteria having a nitrifying action remain to be discovered in the open sea.

The precipitation of calcium carbonate in the sea by bacterial agency is apparently a line of investigation that has not previously been suggested or followed. Both Baur (1) and Gran (9) made use of calcium salts in their culture solutions in order to obviate the great increase in alkalinity that resulted if potassium or sodium salts were used, but they have not called attention to, or apparently realized, the probable significance of this precipitation of calcium carbonate by bacterial agency as an important factor in the formation of various sedimentary calcareous rocks in tropical seas.

The subject of the precipitation of calcium carbonate in sea-water has been dealt with by Murray and Irvine (14) in 1889 and again by Murray (Sir John) and Hjort (13) in 1912, and they ascribe the precipitation to the interaction of ammonium carbonate, derived as an ultimate product of the decomposition of nitrogenous organic matter, with the calcium sulphate present in sea-water, according to the equation

$$(NH_4)_2CO_3 + CaSO_4 = CaCO_3 + (NH_4)_2SO_4$$

Expressed in the terms of the ionic hypothesis, this reaction can be explained by the statement that $CaCO_3$ must be precipitated when the product of the concentration of its ions Ca and CO_3 exceeds a certain limit; an increase in the concentration of CO_3 ions is produced by the advent of $(NH_4)_2CO_3$, which is partially ionized into NH_4 and CO_3, and hence the product of the concentrations of Ca and CO_3 ions is increased and $CaCO_3$ is thrown out of solution.

Though this reaction has been conclusively shown to occur under experimental conditions, where nitrogenous organic matter has been allowed to putrify for some time in sea-water, yet it is obvious that its effect must be purely local and must be confined to the immediate neighborhood of the decaying organic body which gives rise to the formation of $(NH_4)_2CO_3$.

In this paper the precipitation of $CaCO_3$ in an unorganized state alone is dealt with. The formation of the calcareous skeletons, tests, and shells of animals, and the skeletons and platelets of algæ, which play an immensely important part in the constitution of marine bottom-deposits, is beyond the scope of this work.

DESCRIPTION OF APPARATUS.

In 1911 the apparatus at my disposal was of a somewhat primitive nature, as it is difficult to know beforehand exactly what gear will be necessary in a new field of work. In 1912 a more complete outfit was available, and the Carnegie Institution's yacht *Anton Dohrn* was especially fitted for my requirements.

For deep-sea work the motor trawl winch was modified so as to carry fine sounding-wire, and a derrick was rigged aft, projecting over the stern of the boat, over which the wire was led. The motor winch is sunk below the level of the deck, a commendable arrangement, as it can be covered over with hatches when not in use and so affords great economy of deck space, and also has the advantage of bringing the weight of the winch nearer the water-line and avoiding the unstability that may be caused when a heavy winch is fixed on deck.

The sounding-wire was 2.2 mm. in diameter and consisted of four strands of eight wires each, made of high-tensile steel; the breaking strain was given as 400 pounds, but in practice I have no hesitation in saying that it far exceeded this figure. The wire was very difficult to kink, and did not show any tendency to untwist or permanently stretch under a tension of about 350 pounds; it proved in every way satisfactory.

For measuring the length of wire run out, one of the fathom-measuring sheaves made by the Telegraph Construction.and Maintenance Company of London was used. This consisted of a sheave containing a steel wheel about 12 inches in diameter, grooved for and made especially to fit the wire; the length of wire run out is measured by the number of turns of the wheel indicated by a dial on the side of the sheave. The dial has two hands showing fathoms and hundreds of fathoms; the hands revolve backwards on winding in the wire, and so again register zero when the sounding is completed. The axle of the wheel revolves on simple bearings, avoiding thus the slight inaccuracy if ball bearings are employed.

Samples of the bottom were obtained with one of the "snapper rods" disengaging an iron 30-pound weight on touching the bottom. This consisted of two brass jaws closed by a strong spring, and kept apart by a trigger; on touching the bottom the trigger was released and the jaws closed on a sample of the bottom; at the same time the 30-pound weight, which was only held in position through the tension of its own weight, was disengaged as soon as the tension was relieved on touching the bottom, and so was left behind as the wire was reeled in.

In order to tell the depth at which bottom was sounded the wire was led through a pulley connected with a spring balance, which accordingly registered the tension of the wire. On touching the bottom the decrease in tension due to the release of the weight was shown on the dial of the balance. This arrangement was not satisfactory in rough weather, as the rolling of the yacht caused such varying tensions on the wire that it was not always possible to tell the exact depth at which the weight was disengaged.

For obtaining samples of water for bacterial analysis a special water-bottle was designed by Mr. D. J. Matthews. This apparatus is described in detail by Mr. Matthews (12) in vol. IX, No. 4, of the Journal of the Marine Biological Association of the United Kingdom, so only a brief account of it will be given here.

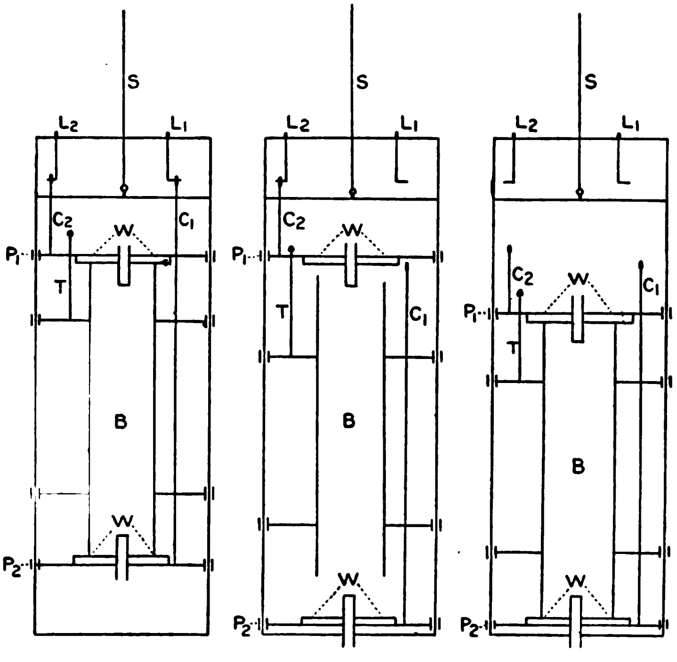
The apparatus employed by previous workers for obtaining samples of water from the deep sea for bacteriological examination has either consisted of some sort of water-bottle made of metal, or else of exhausted glass bulbs with a neck drawn out into a capillary tube which could be broken off at the depth from which a sample was desired. The use of exhausted glass bulbs presents considerable difficulties for depths as great as 800 fathoms; the bulbs must be strong and very thoroughly annealed, as otherwise the slight shock caused by breaking the capillary neck is liable under the great pressure to make the bulb fly into small fragments; another great disadvantage is the strong probability that the sudden reduction in pressure, to which the water is exposed as it enters the bulb, would immediately kill any bacteria in the water. The employment of a metal water-bottle seemed undesirable in view of the bactericidal action of metals. In order to settle this point some test experiments were made with various metals to see if a suitable one could be found. 100 c.c. of water from the laboratory tanks at Plymouth, diluted 1 in 100 with sterile sea-water, was exposed for 6 hours to the action of about 2 square inches of the various metals, with the following results:

Metal.	No. of plates.	No. of colonies of bacteria developing from 1 c.c. after plating on peptone agar, counted after 10 days.
Aluminium bronze...	1	0
" " ...	2	0
" " ...	3	0
Pure copper foil.....	1	1
" " "	2	0
" " "	3	0
Brass..............	1	0
"	2	0
"	3	1
Pure nickel.........	1	17
" "	2	12
" "	3	8
Silver (coins).......	1	3
"	2	2
"	3	4
Control experiment..	1	512
" " ..	2	560
" " ..	3	480

It is thus obvious that none of these metals are suitable for the work, and probably the only metal that could be used would be platinum, which would be prohibitive on account of the expense.

In order to overcome these difficulties, a water-bottle on a new principle was designed for me by Mr. Matthews. A purely diagrammatic representation of its method of working is shown in figure 1.

The container of the bottle consisted of a strong glass cylinder *B*, holding about 250 c.c.; this was closed at each end by thick rubber washers *W*, *W*, through the center of which a short piece of thin-walled rubber tubing passed, the tubing being sealed at the end within the cylinder. The washers were carried by the plates P_1 and P_2. The cylinder was carried in a skeleton frame, and by sending down two messengers along the sounding wire *S* it could be first opened and then closed at any required depth. The whole apparatus was first sterilized by steaming in a "Koch," and then the cylinder was completely filled with 95 per cent alcohol; the washers were kept tight on the ends of the cylinder by strong springs (not shown in the diagram) so that no leakage occurred and the various parts of the apparatus were in the position shown in figure 1, *A*. When the apparatus has been lowered to the required depth, the first messenger is sent down, this hitting the lever L_1, releasing the connecting rod C_1 and allowing the plate P_2 and the cylinder *B* to fall into the position shown in figure 1, *B*, the cylinder being prevented from falling on to P_2 by the check rod *T*, which falls through P_1 until caught by the knob at its extremity. The cylinder is thus opened at each end by the first messenger, and the alcohol being of lower specific gravity than sea-water, diffuses out almost instantaneously, causing an upward flow of water through the cylinder. On sending down the second messenger, which hits the lever L_2, the connecting rod C_2 was released, and by means of strong springs the plate P_1 was forced down on to the top of the cylinder, which at the same time fell on to the plate P_2, and thus the cylinder with its sample of water was tightly closed at each end by the rubber washers, the position of the parts being that shown in figure 1, *C*.



A *B* *C*

Fig. 1.

The washers with their attached pieces of thin rubber tubing had sufficient capability of bulging inwards to allow for the contraction of the alcohol due to the low temperature at any considerable depth, and to its compressibility being greater than that of sea-water; and similarly the expansion of the sample of water, as the apparatus was hauled up, was compensated for by the elasticity of the thin-walled rubber tubing. It is obvious that even had a slight amount of leakage occurred, a leakage inward during the descent of the apparatus would not vitiate the results, as bacteria would promptly be killed in the 95 per cent alcohol, and similarly on hauling up, the leakage, if any, would be outward, due to the expansion of the sample through the regularly increasing temperature and decreasing pressure, so that the sample would not be contaminated by any of the surface layers through which it was hauled. There was, however, no reason to suppose that any leakage occurred, and it appears that the expansibility of the rubber washers and tubing was sufficient to allow for the small changes in bulk of the fluids within the cylinder after the first sterilization by steaming. The action of the alcohol was relied on for sterilization between successive samples, and both experimentally and in practice this method was found to be absolutely safe, as all the marine bacteria are very readily killed by alcohol, and they do not form resistant spores.

After the collection of a sample it was siphoned off into a sterilized glass bottle by means of a sterilized length of rubber tubing; this method was considered preferable to any arrangement of taps leading from the collecting cylinder, owing to the difficulties of cleaning and sterilization which would be involved. Part of the sample was also siphoned off into bottles which were returned to Plymouth for analysis for salinity; these bottles had previously been thoroughly washed, rinsed with several changes of distilled water, and then dried in an oven; they were corked with rubber stoppers.

It was found in practice that this design of water-bottle worked extremely well and gave very little trouble. It is to be noted that the sample of water collected is only kept in contact with rubber and glass throughout, so that the bactericidal action of metals is avoided.

Surface samples of water were taken in wide-mouthed stoppered bottles, holding about 12 ounces; the samples were always taken from the bow of the boat when moving ahead, in order to avoid any possible contamination from the sides of the boat.

Some samples from depths up to 80 fathoms were collected off the Tortugas in 1911 in retort-shaped glass flasks of about 300 c.c. capacity, with narrow, recurved, and long-drawn-out necks. These were sterilized, exhausted, and sealed; they were then lowered in an apparatus in which the extremity of the neck could be broken off at any desired depth by sending a messenger down the sounding-wire, when the flasks became completely filled with water. After hauling up, a little water was shaken from the neck, and it was then sealed with the blowpipe. This method avoids risk of contamination from more superficial layers of water as the apparatus is drawn up, since the changes in pressure and temperature as it ascends tend to cause a continuous outflow through the narrow neck until the surface is reached.

A somewhat similar apparatus was used for obtaining deep samples from the station 70 miles west of Ushant, but the glass bulbs were smaller and the tube leading from them was bent at right angles to itself. Considerable difficulty was caused by the breaking of the tube owing to the force of the inrushing stream of water impinging on the wall where it was bent at right angles.

If this form of apparatus is used, all sharp angles in the inlet tube should be avoided, and it should be so arranged that the inrushing stream of water spreads itself in a fan-shaped manner over the sides of the bulb, but I do not consider that any form of exhausted glass flask is suitable even for depths as small as 80 fathoms.

In Jamaica no apparatus for obtaining deep samples was available, so the primitive method of lowering a sterilized stoppered bottle with a string tied to the stopper was employed. At the required depth the stopper was pulled out until the bottle was nearly full and then allowed to fall back in place. This method can only be used for very shallow depths, as the pressure of the water at greater depths makes it impossible to withdraw the stopper. A source of error is also introduced in that the inrushing water passes in close proximity to the stopper and its attachment, and may carry in bacteria which have adhered to them when passing through the surface layers.

Temperature records were obtained in the Bahamas by means of deep-sea reversing thermometers specially made by Messrs. Negretti & Zambra of London. They were tested up to a pressure of 3 tons to the square inch at the National Physical Laboratory at Teddington, England, and a table of temperature corrections was furnished for each instrument by the same institution. These reversing thermometers differ from ordinary thermometers in having a constriction and S-shaped dilatation immediately above the main bulb, with a somewhat large secondary bulb at the upper end of the stem. The graduations are reversed, so that the lowest temperature is marked near the top of the capillary portion. On turning the thermometer upside down, the mercury thread breaks at the constriction and fills the small bulb at the end of the capillary and also part of the capillary itself. The thermometer is read in the reversed position, and when certain corrections have been applied the temperature at which the thermometer was reversed is recorded. The effect of the pressure of the water is avoided by having the thermometer sealed in an outer glass case. The lower end of this case is partially filled with mercury, in which the bulb of the thermometer is immersed, thus allowing for rapid conduction of heat between the mercury in the thermometer bulb and the surrounding water. An auxiliary thermometer was sealed up in the same outer case as the reverser, so that the temperature at which the actual reading was taken could also be recorded. In order to calculate the correction that must be applied to the temperature registered by the reverser, three factors must be known:

(*a*) Temperature of thermometer at moment of reading.
(*b*) Kind of glass of which it is made.
(*c*) Volume (expressed in degrees of the stem) of the secondary bulb and the portion of the stem below the 0° graduation.

Of these *a* is given by the auxiliary thermometer, and *b* and *c* were engraved on the back of the stem of each reversing thermometer. All of the thermometers were made of the glass known as Jena^{er} 16 III, and the apparent dilatation of mercury in this glass is $\frac{1}{6300}$. The correction to be applied to the reading of the reverser is given by the formula $\frac{(V^\circ+T)(T-t)}{6,300}$, where T = the temperature registered by the reverser, t = the temperature shown by the auxiliary thermometer at the moment of reading, and V = the volume (expressed in degrees of the stem) of the secondary bulb and the portion of the stem below the 0° mark of the reverser.

The thermometers were mounted in pairs in simple metal cases, and were attached just below the water-bottle. They were suspended in a vertical position by a catch forming part of the water-bottle; this was released by the first messenger, when the thermometers fell by their own weight and so reversed, and were hauled up in this position. This simple arrangement proved quite as satisfactory as any of the more complicated reversing frames which are generally in use.

CULTURE MEDIA AND METHODS.

The culture media employed for isolating and counting the bacteria in plate cultures were the following.

I. Peptone agar: Peptone, ~~2.0~~ 5 grams; potassium nitrate (~~KNO~~ Cn. $_3$), 0.5 gram; sea-water, 1,000.0 c.c.; agar agar, 18.0 grams.

In the earlier work less agar was used, but eventually it was found more convenient to use a stiffer jelly, and this did not appear to appreciably hinder the growth of the bacteria.

II. Potassium malate agar: Potassium malate $\left(C_2H_3(OH)\left\langle\begin{matrix}COOK\\COOK\end{matrix}\right.\right)$, 1.0 gram; sodium phosphate (Na_2HPO_4, $12H_2O$), 0.25 gram; potassium nitrate (KNO_3), 0.5 gram; sea-water, 1,000.0 c.c.; agar agar, 12.0 grams.

The medium was only filtered through glass-wool, so that a very slight floccular precipitate of calcium phosphate was retained.

III. Peptone gelatin: Peptone, 2.0 grams; potassium nitrate (KNO_3), 0.5 gram; sea-water, 1,000.0 c.c.; gelatin, 150.0 grams.

This medium was used only at the Tortugas; it was necessary to keep it cooled with ice to about 20° C., as the temperature of the laboratory sometimes rose as high as 37° C. and gelatin media will not remain solid at these temperatures.

The following fluid media were used:

I. Gran's medium (modified): Potassium nitrate (KNO_3), 0.5 gram; sodium phosphate (Na_2HPO_4, $12H_2O$), 0.25 gram; calcium malate $\left(C_2H_3(OH)\left\langle\begin{matrix}COO\\COO\end{matrix}\right\rangle Ca\right)$, about 5.0 grams; sea-water, 1,000.0 c.c.

Calcium malate is only slightly soluble in water, so can be added in excess. Gran used distilled water and added 30 gm. sodium chloride per liter, but in these experiments sea-water has been used instead.

II. Calcium succinate medium: Calcium succinate $\left(C_2H_4\left\langle\begin{matrix}COO\\COO\end{matrix}\right\rangle Ca\right)$, 2.0 grams; potassium nitrate (KNO_3), 0.5 gram; sodium phosphate (Na_2HPO_4, $12H_2O$), 0.25 gram; sea-water, 1,000.0 c.c.

This medium was boiled and filtered before sterilization to remove the slight precipitate of calcium phosphate. It was found that this medium with the addition of the phosphate gave a more vigorous growth than if it was omitted.

III. Calcium acetate medium: Calcium acetate ($Ca(CH_3COO)_2$), 5.0 grams; sodium phosphate (Na_2HPO_4, $12H_2O$), 0.25 gram; potassium nitrate (KNO_3), 0.5 gram; sea-water, 1,000.0 c.c.

Boiled and filtered before sterilization to remove precipitate of phosphate.

IV. Peptone calcium acetate medium: Calcium acetate ($Ca(CH_3COO)_2$), 5.0 grams; peptone (Witte's), 0.2 gram; potassium nitrate (KNO_3), 0.5 gram; sea-water, 1,000.0 c.c.

Media II, III, and IV were also made up with the addition of 0.2 gm. of magnesium tartrate per 1,000 c.c.

The fluid media were made up in 1,500 c.c. resistance glass flasks, and 1,000 c.c. of medium were used for each culture.

For other purposes a simple solution of peptone in sea-water was employed (2 gm. to 1,000 c.c.), and media were also used consisting of this peptone solution with the addition of 0.5 per cent of various carbohydrates, such as cane sugar, dextrose, lævulose, mannite, lactose, etc., with sufficient neutral red solution to color them, in order to test the acid-forming properties of the bacteria in the presence of carbohydrates.

The ordinary "Koch" steam sterilizer and iron oven for dry-heat sterilization were used, and gasoline cooking stoves were found to be the most satisfactory source of heat. It was found an advantage to use Petri dishes with porous earthenware covers which enabled the water of condensation to evaporate partially; the evaporation could be checked at any time by covering the dishes with a bell jar lined with wet filter paper. It was usually found necessary to keep all cultures on tables with their feet standing in dishes of kerosene, in order to prevent the attacks of ants and other insects. In all other respects ordinary bacteriological routine was followed, and the methods need not be further particularized here.

The reduction of the nitrate to a nitrite in fluid culture media was tested for by the addition of 5 c.c. of 10 per cent sulphuric acid and 2 c.c. of a 1 per cent solution of metaphenylene diamine hydrochloride to 25 c.c. of the culture. The production of a brown coloration (due to the formation of Bismarck brown) is an indication of the presence of a nitrite, and is an extremely delicate reaction.

The diphenylamine and brucine sulphate reactions were also used when testing for the presence of nitrates.

The formation of ammonia was tested for by the addition of 5 c.c. of 10 per cent potassium hydrate and 5 c.c. of Nessler's reagent; the white precipitate formed on the addition of the potassium hydrate does not appreciably interfere with the test, though it renders it somewhat less delicate.

Under expeditionary conditions, and in the absence of the somewhat elaborate apparatus that would be necessary in order to estimate chemically the amount of denitrification in cultures, it was only possible to compare the rate of denitrification in different cultures by noting the time taken for the first appearance of the nitrite reaction and the time taken for all trace of nitrite or nitrate to disappear. It seems that the rate of denitrification in culture media inoculated with equal volumes of samples of sea-water must be a function of the number of bacteria in the sample, the temperature at which the cultures are grown, and the specific power of denitrification of the individual species of bacteria. Considering the rapid multiplication of bacteria when the food supply is plentiful, up to a maximum determined chiefly by the accumulation of the waste products of their own metabolism, it appears that the factor of the number of bacteria in the sample may be neglected within the limits of these experiments. For example, the number of bacteria in 1,000 c.c. of Gran's medium at the end of 24 hours would probably be much the same whether it were inoculated from a sample containing 8 or 16 bacteria per 1 c.c.; similarly it was a matter of experience that the first trace of nitrite formation was observable at about the same time, whether 5 or 10 c.c. of a given sample had been used for inoculation.

Consequently it would appear that for purposes of comparison, and within the limits of the experiments described, if the temperature be the same for the cultures compared, the rate of denitrification is a measure of the specific denitrifying power of the particular species of bacteria.

In the work on the bacterial precipitation of calcium carbonate, the precipitate (which was often so fine as to tend to remain in suspension) was usually obtained by centrifuging. It was either preserved in small bottles with some of the culture fluid, or else washed first with distilled water and then with absolute alcohol, and finally allowed to dry. These precipitates were sent to Dr. F. E. Wright, of the Geophysical Laboratory of the Carnegie Institution of Washington, who with great kindness reported on their mineralogical properties.

INVESTIGATION OF SAMPLES OF SEA-WATER TAKEN OFF PORT ROYAL, JAMAICA.

The work at Port Royal was done in May 1911, but was of a very preliminary nature. It was necessary to depend on a sailing-boat for obtaining the samples; yet, owing to the remarkable regularity with which an on-shore wind springs up every morning, but little difficulty was experienced from this cause. No apparatus for obtaining deep samples was available, but samples were taken from a depth of 6 fathoms by means of a bottle from which the stopper was pulled by a line and then allowed to fall back into place. A measurement of the rate of denitrification in fluid culture media inoculated with samples of sea-water was made, but isolation of the bacteria on solid media was not attempted. The following method was employed:

Samples of sea-water were collected in sterilized stoppered bottles from the surface and from depths of 3 and 6 fathoms from positions about 5 miles from shore, where, from a consideration of the wind and tide, the water was probably under truly oceanic conditions and unaffected by the neighboring land. 10 c.c. of these samples were added to 1,000 c.c. of Gran's medium. The cultures were kept in a moderate light and the temperature varied from 25° to 31.5° C. The average temperature during the growth of each culture was noted.

In a typical culture made from surface water, and for which the average temperature was 29° C., the first indication of the formation of a nitrite, as given by the metaphenylene diamine reaction, appeared after 27 hours; after 38 hours the brown color produced in this reaction was very intense, the culture became cloudy, and on testing with Nessler's reagent slight ammonia formation was apparent. After 48 hours the culture became very cloudy and a scum of bacterial growth developed; the nitrite and ammonia reactions remained unaltered. After 63 hours the nitrite reaction was somewhat less marked, the ammonia reaction was unaltered, and bubbles of gas began to appear. After 72 hours many bubbles of gas were being produced, and the nitrite and ammonia reactions were very slight. After 86 hours the bubbling had ceased, and no nitrite or ammonia was present in the culture. Testing the culture for nitrates by the brucine and diphenylamine reactions then showed that no nitrate was left in the solution.

In the absence of a gas-analysis apparatus the nature of the gas evolved could not be determined, but considering that it was non-inflammable, did not turn lime-water milky, and that the nitrate originally present had been destroyed, it seems strongly probable that this gas was pure nitrogen. Thus in 86 hours 0.5 gm. of potassium nitrate had been decomposed by bacterial growth. If a further 0.5 gm. of potassium nitrate were then added, it was rapidly decomposed, and this could be repeated many times until the other constituents of the culture medium were used up.

It was found that the rate of denitrification varied somewhat with the temperature, and that in cultures kept at a temperature of between 10° and 12° C. no growth or denitrification occurred. The denitrification was always more rapid in cultures from water taken from a depth of 3 or 6 fathoms than from the surface. It was also more rapid with samples taken from the thick muddy water of a mangrove swamp, where organic matter was plentiful.

The bacteria present in the cultures were very minute, actively motile bacilli with rounded ends.

An abstract of the behavior of a few of the cultures is given below.

1. Sample collected 5 miles south of Port Royal, wind southeast, force 4, tide rising. Sample taken from surface. 1,000 c.c. of Gran's medium was inoculated with 10 c.c. of sample.

After 20 hours a slight cloud developed in the culture and faint nitrite reactions were given.

After 36 hours a dense cloud developed in the culture and strong nitrite reactions were given.

After 60 hours a dense cloud and scum developed in the culture and strong nitrite and faint ammonia reactions were given.

After 70 hours a dense cloud, scum, and bubbles developed in the culture and faint nitrite and faint ammonia reactions were given.

After 84 hours culture was less cloudy, with much scum, no nitrite or nitrate reaction, very faint ammonia.

Average temperature at which the culture was grown, 30° C.

2. Sample collected from same spot under similar conditions, from a depth of 3 fathoms. 1,000 c.c. Gran's medium was inoculated with 10 c.c. of sample.

After 20 hours a slight cloud developed in the culture and faint nitrite reaction was given.

After 27 hours a denser cloud developed in the culture and strong nitrite and faint ammonia reactions were given.

After 38 hours a dense cloud and scum developed in the culture and strong nitrite and faint ammonia reactions were given.

After 48 hours a dense cloud and scum developed in the culture and moderate nitrite and faint ammonia reactions were given.

After 63 hours a moderate cloud, thick scum, and bubbles developed in the culture and faint nitrite and faint ammonia reactions were given.

After 72 hours a slight cloud and thick scum, no nitrite or nitrate, and very faint ammonia reaction.

Average temperature at which the culture was grown, 29° C.

3. Sample collected from a spot 6 miles south of Port Royal, wind ESE., force 4, high tide (slack). Taken from surface. 1,000 c.c. Gran's medium was inoculated with 10 c.c. of the sample.

After 20 hours a slight cloud developed in the culture, no nitrite reaction was given.

After 27 hours a slight cloud developed in the culture, faint nitrite reaction was given.

After 38 hours a dense cloud developed in the culture, strong nitrite and faint ammonia reactions were given.

After 48 hours a dense cloud and scum developed in the culture, strong nitrite and faint ammonia reactions were given.

After 63 hours a dense cloud and scum developed in the culture, moderate nitrite and faint ammonia reactions were given.

After 72 hours a moderate cloud, scum, and bubbles developed in the culture, very slight nitrite and faint ammonia reactions were given.

After 86 hours a moderate cloud and scum, no nitrite or nitrate and very faint ammonia reaction.

Average temperature at which the culture was grown, 29° C.

4. Sample taken from surface water of the large mangrove swamp lying northwest of Port Henderson. 1,000 c.c. of Gran's medium inoculated with 10 c.c. of sample.

After 20 hours no cloud or nitrite reaction.

After 24 hours slight cloud and slight nitrite reaction.

After 40 hours strong cloud and scum, strong nitrite and slight ammonia reaction.

After 75 hours cloud, scum, and bubbles, no nitrite or nitrate and slight ammonia reaction.

Average temperature at which the culture was kept, 30° C.

5. Subculture from culture (1). 1,000 c.c. Gran's medium inoculated with 5 c.c. of culture (1), and kept at a temperature of 10° C. to 12° C. by means of ice.

After 100 hours the culture was quite clear and gave no nitrite reaction. It was then removed from the ice and kept at the room temperature, which averaged 30° C.

After 107 hours a dense cloud developed in the culture and strong nitrite and faint ammonia reactions were given.

After 120 hours a dense cloud, scum, and bubbles developed in the culture and moderate nitrite and faint ammonia reactions were given.

After 131 hours a faint cloud, scum, and bubbles developed in the culture; very faint nitrite and faint ammonia reactions were given.

After 146 hours a faint cloud and scum, no nitrite or nitrate, and very slight ammonia reactions were given.

Twenty cultures were made from samples of water taken well out to sea from Port Royal, and the process of denitrification followed through with each. All gave very similar and consistent results, but the rate of denitrification decreased rapidly with the temperature at which the cultures were grown; thus at an average temperature of 27° C. the first trace of the nitrite reaction appeared after about 40 hours, and denitrification was complete after about 100 hours.

The results of precisely similar experiments that I made with samples of water taken from the English Channel near Plymouth in the autumn of 1909 showed that there the process of denitrification was very much slower, and was never complete at the room temperature (17° C.). The first trace of the formation of a nitrite in cultures in the modified Gran's medium, as detected by the metaphenylene diamine reaction, occurred about the fifth day, and a large proportion of the nitrite and nitrate always remained, even in the oldest cultures. In similar cultures incubated at 30° C., denitrification was complete by the eighth day at earliest, but uniformly consistent results were not obtained, as in some of the cultures complete denitrification was never obtained, even after several months.

It would thus appear that even under similar temperature conditions the marine bacteria in the seas off Jamaica are much more active in causing denitrification than those found in the English Channel, and since the rate of denitrification is a function of the temperature, it follows all the more that the destruction of nitrates by bacterial agency in the seas around Jamaica must be far in excess of that occurring in the cooler waters of the English Channel.

THE INVESTIGATION OF SAMPLES OF SEA-WATER TAKEN AROUND THE DRY TORTUGAS.

The Dry Tortugas consist of a group of eight small keys, the largest of which (Loggerhead Key) is only about three-quarters of a mile long by one-eighth of a mile wide. They are situated about 150 miles from the mainland of Florida, and form the extreme western end of the chain of the Florida Keys. The 100-fathom line lies some 30 miles to the south and southwest of the islands, and then trends round in a northwest direction; beyond the 100-fathom line the depth increases with moderate rapidity until depths of from 1,000 to 1,400 fathoms are reached. To the east, northeast, and north as far as the coast of Florida, the water is shallow, the soundings showing from 20 to 30 fathoms in most places. Beyond the 100-fathom line to the southward the influence of the Gulf Stream begins to make itself felt, though the region of maximum current velocity here lies nearer the coast of Cuba. The Tortugas Keys are of purely coral formation, consisting entirely of broken shell and coral sand, with no soil. The greatest elevations are the hurricane ridges, which are not more than 15 feet above sea-level, and during a hurricane the islands are sometimes completely submerged. There is no vegetation on the smaller keys, but Loggerhead Key, on which the Marine Biological Laboratory of the Carnegie Institution of Washington is situated, is partially covered with a growth of bushes and coarse grass. There is no fresh-water supply on the islands.

From these considerations it is obvious that the risk of contamination of samples of sea-water taken a few miles from the Keys through land bacteria is very small, and that such samples may be taken as being truly oceanic.

The motor yacht *Anton Dohrn* and smaller motor boats made the collection of samples an easy matter, and the well-equipped laboratory made possible fuller investigations than those attempted in Jamaica.

A number of cultures were made in Gran's medium under conditions exactly comparable to those made at Port Royal, and the rate at which the process of denitrification proceeded was observed. The results agreed almost exactly with those obtained at Port Royal, so need not be described in detail. It thus seems that the denitrifying power of the bacteria in the seas around the Tortugas is the same as that of those around Jamaica.

Cultures were also made on various solid media, and pure cultures of the various species of bacteria were isolated by plating in Petri dishes with peptone agar. Samples of surface water taken from various positions around Tortugas as far as possible removed from influence of the land, and collected on sunny days, gave an average count of 14 colonies per 1 c.c. of sample. Counts of several plates from the same locality, and from different localities, showed a somewhat remarkable agreement as to the number of colonies present, the highest count ever obtained being 20 and the lowest 8 per 1 c.c. Allowing for experimental error, this shows great uniformity in the distribution of bacteria in the sea around Tortugas.

The colonies appeared to be of two kinds when grown on peptone agar, one much more plentiful than the other. Subcultures made from these colonies in Gran's medium showed that the bacteria forming the most common type of colony produced an active denitrification, while the others grew very slowly in this medium and produced no denitrification.

The characteristics of the denitrifying form are as follows:

On the potassium malate, or peptone agar media, colonies are visible as minute white specks after 6 to 8 hours when the room temperature averages 29.5° C. After about 18 hours the colonies are well developed; they are white in color, circular but with finely irregular outline, and have a granular appearance. Superficial colonies are much elevated at first, but as growth proceeds they spread rapidly over the surface of the agar. Deep colonies remain small, circular, and discrete.

Growth is somewhat more rapid on peptone agar than on the potassium malate agar, and the older colonies develop a brownish tinge in the center when growing on the former medium. On gelatin peptone (5 per cent peptone in sea-water and kept at between 20° and 25° C. to insure the medium remaining solid), growth was very slow; in stab cultures growth proceeded slowly from the surface downwards, leaving a funnel-shaped depression of liquefied gelatin.

Acid formation occurs in dextrose, lævulose, mannite, and cane sugar, but not in lactose media.

Growth is inhibited at a temperature of 10° C., but takes place slowly at 15° C.

Growth is much retarded by exposure to bright sunlight, but the bacteria are not killed by a 10 hours' exposure.

The bacteria are facultative anaërobes, but growth under anaërobic conditions is very slow.

In Gran's medium growth is rapid, but no growth occurs if the potassium nitrate be omitted, or if the calcium malate be replaced by calcium carbonate. Growth in a pure solution of peptone in sea-water is very slight, but becomes abundant if potassium nitrate be added, when denitrification quickly ensues. The most rapid growth was produced in sea-water containing 2 per cent peptone, 1 per cent potassium malate, and 0.5 per cent potassium nitrate, and in this clear medium a slight floccular precipitate, presumably of calcium salts derived from the sea-water, was soon formed. Growth was also rapid at first in a solution of 5 per cent potassium malate and 0.5 per cent potassium nitrate in sea-water, but growth apparently ceased in this medium after a few days and denitrification was never complete; a slight precipitation occurred and the solution was found to have very definitely increased in alkalinity.

This bacterium does not appear to have been previously described, and I propose for it the name of *Bacterium calcis*, owing to its power of precipitating calcium carbonate from solutions of calcium salts. This point will be dealt with later in the paper.

The characteristics of the scarcer non-denitrifying form of bacterium found on the agar plates are as follows:

Growth on the potassium malate agar medium is very slow and indefinite. On peptone agar growth is somewhat slower than in the case of the denitrifying form. On the surface, circular cream-colored colonies are formed, having a brownish center; the edges are smooth and regular, and the colony remains discrete and does not tend to spread over the surface. The deep colonies are smaller and usually ovoid in shape, and of a somewhat darker color than those on the surface.

No growth was obtained on gelatin media.

Acid formation as shown by the neutral red reaction occurs in dextrose and lævulose, but not in cane sugar, lactose, or mannite media.

Growth takes place slowly at 10° C. No visible growth occurred at 0° C., but cultures were not killed by 24 hours' exposure to this temperature.

Growth is retarded by light, and cultures are killed by 4 hours' exposure to bright sunlight.

The bacterium is a strict aërobe.

Free growth takes place in Gran's medium, but develops much slower than in the case of the denitrifying form. No growth occurs if the potassium nitrate be omitted entirely, but takes place freely if a mere trace in excess of that normally present in the sea-water be added, though no denitrification results. Attempts were made to discover whether this bacterium had any nitrifying or denitrifying action in various culture media, but uniformly negative results were obtained. Nitrites were neither oxidized to nitrates nor reduced to ammonia or free nitrogen, and ammonia salts were unaffected. No growth was obtained in any culture medium that did not contain at least a trace of nitrates, so it was not practicable to ascertain whether the bacterium had a nitrifying action without the necessary facilities for quantitative work.

On one occasion samples were obtained from various depths up to 90 fathoms at a point near the Gulf Stream region, 25 miles south of Tortugas. Exhausted glass flasks with capillary necks which could be broken off at the required depth were used for the purpose.

These samples were plated in the peptone agar medium and counted, with the following average results:

Depth in fathoms.	Denitrifying orms (*Bacterium calcis*).	Non-denitrifying forms.	No. of colonies developing from 1 c.c. of sample.
0	9	2	11
10	25	4	29
40	2	2	4
60	5	3	8
90	5	6	11

These figures are probably not very reliable, especially for the greater depths, since it is possible that many of the bacteria were killed by the sudden reduction of pressure to which they were exposed as the water entered the exhausted bulb.

INVESTIGATION OF SAMPLES FROM A POINT 70 MILES WEST OF USHANT ISLAND, FRANCE.

This spot was chosen as it is sufficiently far out in the Atlantic to be largely removed from the influence of the English Channel water. The object was to investigate truly oceanic bacteria, and previous work in 1909 had shown that the bacterial flora of the Channel water was relatively very complicated, probably owing to the presence of littoral forms. The Marine Biological Association of the United Kingdom very kindly sent their steamship *Oithona* from Plymouth for this work, and gave me every facility both on board and in their laboratory. As in Tortugas, the deep samples were collected in exhausted glass flasks, and accordingly, as previously explained, the results obtained from the deep samples can not be considered to possess any very great degree of accuracy.

Attempts were made to plate the sample in peptone agar on board the boat, but the result was not satisfactory, as owing to the motion of the boat the jelly set in irregular waves and lumps. Consequently the samples were kept on ice, and cultures were made from them at Plymouth 24 hours after collection. It is clear that in future attempts to make plate cultures on board a small boat in rough weather, a very delicately swung table will be necessary, or else the roll-tube culture method must be employed.

Three plates on peptone agar were made from each sample, 1 c.c. of the sample being used for each plate. The plates were kept at the room temperature, averaging about 20° C., and the colonies were well developed after 48 hours; they appeared to be all of one kind. A count gave the following results:

Depth in fathoms.	No. of colonies developing from 1 c.c. of sample.
0	7
10	9
20	6
30	5
50	6
70	30
80	20

The increase in the number of colonies at 70 and 80 fathoms is somewhat remarkable, but no conclusions in this respect can be drawn from one series of observations.

The cultural characteristics of this bacterium are as follows:

On peptone agar, after about 36 hours at 20° C., the colonies are white in color, circular, with a finely serrated outline and a coarsely granular appearance. Superficial colonies grow very rapidly, and may spread as a whitish semi-transparent growth of irregular shape over the surface of the agar. The deep colonies remain small, globular, and discrete. In old agar cultures a brownish tinge is developed, and the color may diffuse through the substance of the agar. On gelatin peptone growth was rapid; in stab cultures growth proceeded from the surface downwards, leaving a funnel-shaped depression of liquefied gelatin, and eventually all the gelatin became liquefied.

Acid formation, as shown by the neutral-red reaction, took place in dextrose, mannite, and lævulose, but not in cane sugar or lactose media.

One thousand c.c. of Gran's medium, inoculated on board with 10 c.c. of a surface sample immediately after collection, and kept at an average temperature of about 20° C., showed the first trace of nitrite formation after 70 hours. After 84 hours a very strong nitrite reaction was obtained, and a slight ammonia reaction was given with Nessler's reagent. The process of denitrification, even after the lapse of weeks, did not extend beyond this, and no bubbles of gas were formed. Other experiments made with subcultures from agar and gelatin media gave similar results, so that it appears that this bacterium can not entirely break down nitrates at a temperature of 20° C. The optimum temperature for denitrification produced by this bacterium appears to be about 20° C., as the process was less rapid at average temperatures of 17° C. and 25° C. At a temperature of 32° C., rapid growth took place, but no denitrification resulted.

It should be noted that these temperature observations were only made with subcultures from colonies on peptone agar and peptone gelatin media, and there is reason to believe that the power of denitrification becomes diminished after cultivation on such media. Further and more accurate temperature experiments are required in which the culture medium is directly inoculated with freshly collected samples of water.

This bacterium appears to be closely related to the *Bacterium calcis*, its chief points of difference being:

1. Lesser denitrifying power and lower temperature optimum for denitrification.
2. More rapid growth on gelatin media.
3. Absence of acid formation in media containing cane sugar.

INVESTIGATION OF SAMPLES FROM MARQUESAS KEYS, AND EXPERIMENTAL PRECIPITATION OF CALCIUM CARBONATE BY BACTERIAL AGENCY.

The Marquesas Keys constitute a coral atoll which forms part of the long chain of keys separating the Gulf of Mexico from the Straits of Florida. Within the atoll the water is very shallow and the bottom consists of a fine chalky mud many feet deep. Samples of the water from the lagoon of the atoll were sent to me at Plymouth by post, and examined 14 days after collection.

On plating on peptone agar, an average of 800 colonies per 1 c.c. of the sample were obtained. These colonies appeared to be all of one species, and were identical in appearance and in all cultural characteristics with the *Bacterium calcis* previously described as occurring around the Tortugas.

A suspension of these bacteria from a culture on peptone agar was made in sterile sea-water, and a similar suspension containing roughly the same number of bacteria was made from a third subculture on peptone agar of the bacteria obtained from the station 70 miles west of Ushant. 1 c.c. of each of these suspensions was then added to 1,000 c.c. of the modified Gran's medium. Some of these cultures were kept at an average temperature of 20° C. and others at 32° C., with the following results:

At 20° C. cultures from Marquesas showed trace of nitrite after 45 hours.
At 20° C. cultures from Marquesas gave strong nitrite reaction after 53 hours.
At 20° C. cultures from 70 miles west of Ushant showed trace of nitrite after 140 hours.
At 20° C. cultures from 70 miles west of Ushant showed strong nitrite reaction after 162 hours.

In both cases a slight amount of ammonia was recognizable by Nessler's reagent when the nitrite reaction was strong, but decomposition of the nitrite did not proceed further even after 14 days.

At 32° C. cultures from the Marquesas showed trace of nitrite after 18 hours.
At 32° C. cultures from the Marquesas gave strong nitrite reaction after 22 hours.
At 32° C. cultures from 70 miles west of Ushant never gave nitrite or ammonia reaction.

The cultures from the Marquesas showed a slight amount of ammonia formation, but the decomposition of the nitrite did not proceed further.

THE EFFECTS OF TEMPERATURE UPON TROPICAL MARINE ANIMALS.

By Alfred Goldsborough Mayer.

CONCLUSIONS.

Tropical marine animals commonly live within 5° C. of their temperature of maximum activity and within 10° to 15° C. of their upper death temperature. In marine animals of the temperate or arctic regions a considerable range of temperature above or below the normal produces relatively little difference in their activities, but in tropical forms even a few degrees of heat or cold cause a marked depression in movement. In tropical Scyphomedusæ this depression of movement appears to augment about as the square of the change in temperature from that of the optimum. Thus it is more depressant than one would expect were it due to a simple chemical reaction.

As was shown by Harvey (1911), the rate of nerve-conduction in the subumbrella tissue of *Cassiopea* varies as the increment of temperature, increasing in a linear ratio as the temperature is raised.

Experiments conducted at the Murray Islands, Australian Great Barrier Reef, show that those corals which die at temperatures below 36.5° are killed by being buried 11 hours under the mud, but those which resist 37°, and above, are proportionally less sensitive to the smothering effects of mud and may survive being buried for 30 to 40 hours. This suggests that high temperature produces death by causing asphyxiation, the oxygen of the sea-water being insufficient to sustain the increased metabolic activity of the animal.

OBSERVATIONS AND EXPERIMENTS.

The literature upon the effects of temperature on animal life and activities is both extensive and widely scattered, although Davenport and Castle (1895), in their paper in the "Archiv für Entwicklungsmechanik der Organismen," Bd. 2, have given a list of all previous papers treating of experiments upon the maximum temperature which can be endured by aquatic organisms; and Bachmetjew (1899), in the "Zeitschrift für wissenschaftliche Zoologie," Bd. 66, presents a complete résumé of researches upon the subject of the temperature of insects in relation to that of their surroundings. Also a review of works upon the physiological effects of temperature is given by Demoll and Strohl (1909).

Probably no single factor is a more effective barrier to the extensive geographical range of marine animals than is that of temperature. This fact has long been recognized and there are many striking examples of its

From these experiments it appears that the bacteria from subcultures from the Marquesas have a much greater denitrifying power than those from subcultures from a point 70 miles west of Ushant, and that as the bacteria from the Marquesas appear to be of the same species as those investigated at the Dry Tortugas, their power of causing complete denitrification in Gran's medium has been lost by successive cultivations on peptone agar.

The presence of the thick layers of fine chalky mud within the Marquesas Keys, and elsewhere in many places near the Florida coast, led to a consideration of the possibility of its precipitation by bacterial agency.

Since these bacteria grow freely in Gran's medium, the calcium salt of a simple organic acid is a sufficient source of organic food for them, and it seems probable that they would thrive in sea-water containing the products of decomposing vegetable matter, provided that the nitrate supply and conditions of light and temperature were suitable. Such conditions should be especially well fulfilled by the drainage into the sea of a well-wooded country with a calcareous subsoil, and the soluble organic calcium salts would be precipitated as calcium carbonate by the action of the bacteria. In addition, the elimination of the acid radicle from the nitrate in the process of denitrification, by whatever stages it may occur, must leave the alkaline base free to destroy the normal equilibrium of the salts in sea-water, and by increasing the alkalinity would also result in the precipitation of calcium carbonate.

To test this theory cultures were made in a medium having the following composition:

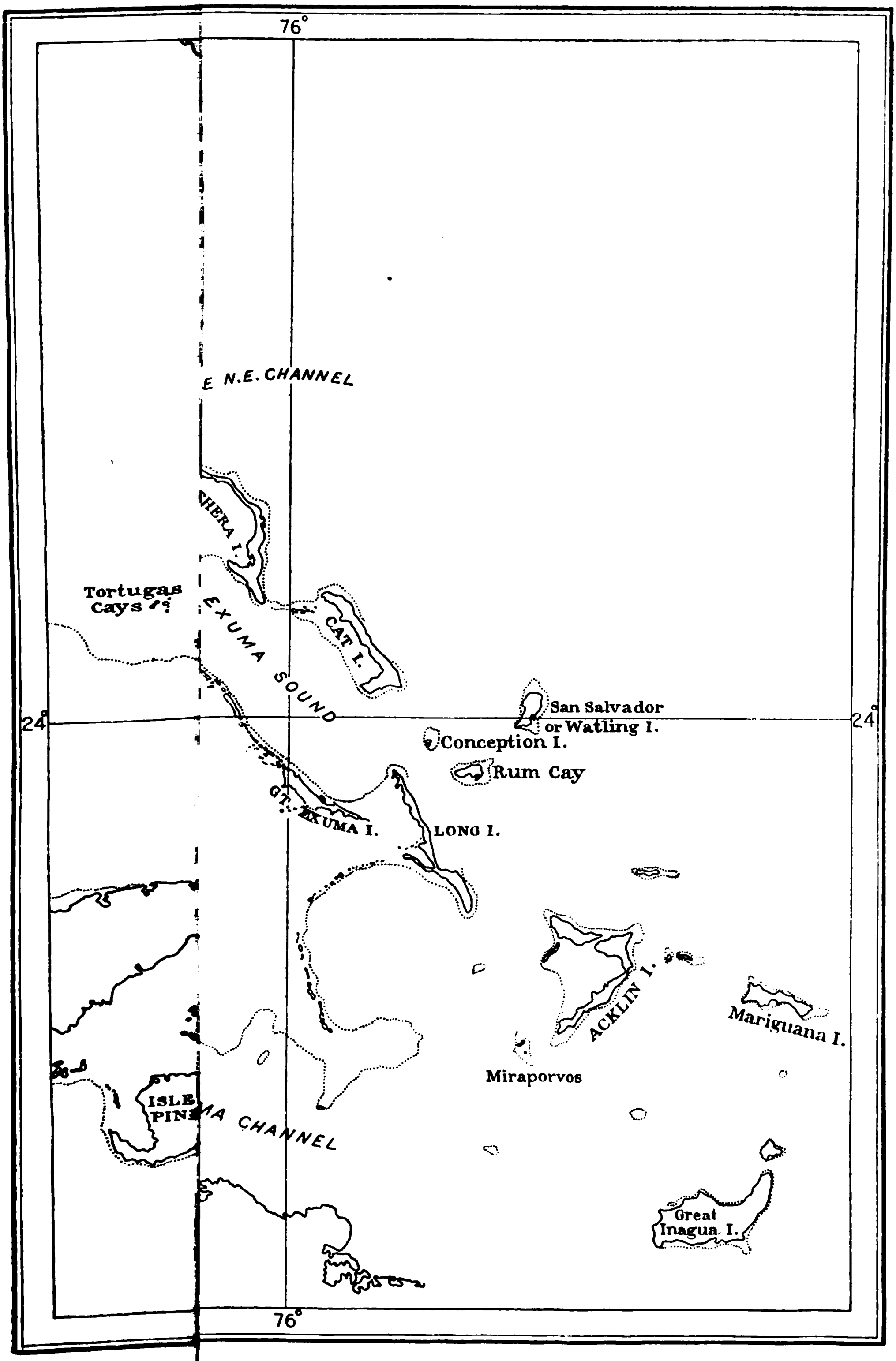
Calcium succinate	2.5 grams
Potassium nitrate	0.5 gram
Sea-water	1,000.0 c.c.

Calcium succinate is soluble in these proportions and the medium is quite clear. Free growth was manifested by the cloudiness of the medium 48 hours after inoculation, and nitrite formation was apparent.

After 96 hours the medium appeared quite milky and this milkiness was due to the presence of exceedingly fine particles of a substance which was soluble in dilute hydrochloric acid with evolution of gas, and was presumably calcium carbonate. In some cultures these particles settled as a definite sediment, but in others the particles were so minute that they showed little tendency to settle and could only be separated with difficulty by centrifugalization. The conditions determining the size of the particles formed could not be ascertained, as the size varied in cultures which were apparently made and grown under identical conditions.

The addition to cultures in which the particles of calcium carbonate were so small as to remain in suspension of any foreign substance, such as finely powdered calcium sulphate or of larger particles of sand, resulted in the aggregation around them of the particles of calcium carbonate, forming a concretion around a central nucleus. These concretions were hard and of

CHART A



. . . . ama Island.

almost crystalline appearance under the microscope, and were soluble in dilute hydrochloric acid, with evolution of bubbles of a gas which, when the operation was performed on a microscopic slide, could be completely absorbed by running in a solution of sodium hydrate under the cover slip. Once this process of concretion has been initiated, it appears to progress independently of the presence of particles which act as nuclei, and a large concretion may often be found having a number of smaller concretions around it, or continued into a chain of small spheres, the whole presenting somewhat the arrangement shown by freely budding yeast cells. The deposition of this form of calcium carbonate also takes place on the sides of the flask, and more especially over any area where the glass is scratched or roughened.

From these results it would seem strongly probable that the layers of fine and unorganized chalky mud found in the Marquesas Keys are being precipitated by the action of the *Bacterium calcis*, and it would seem a reasonable suggestion that similar bacterial action may have played an important part in the formation of chalk and other limestone formations in geologic times. The formation of semi-crystalline concretions around a central nucleus at first seemed to suggest an explanation of the formation of oolite grains, but a mineralogical examination, very kindly made by Dr. Fred. E. Wright, showed that the concretions did not possess that laminated structure characteristic of oolite grains and that their crystalline structure was nearer that of calcite than aragonite.

SOME CONSIDERATIONS ON THE PHYSIOGRAPHY OF THE TONGUE OF THE OCEAN AND ANDROS ISLAND.

The position of the Tongue of the Ocean is shown in Chart A, which includes the greater part of the Bahama group and shows its position relative to Florida and Cuba. The Tongue consists of a long and narrow stretch of deep water, running in a NNW.–SSE. direction, and except at its northern end it is completely surrounded by shallows or land. On the west, for about three-fourths of its length, it is bounded by the coast of Andros Island; southwest of Andros it is separated from Santaren Channel by some 60 miles of shallow water lying over the Great Bahama Bank; to the south it is separated from the Old Bahama Channel by over 50 miles of shallows, averaging not more than 3 fathoms in depth; to the east it is separated from the deep water of Exuma Sound by from 20 to 40 miles of shallow water of from 2 to 3 fathoms in depth, and by the chain of islands and cays extending in a NNW. direction from Great Exuma Island. The mouth of the Tongue of the Ocean lies between New Providence Island on the east and the northern extremity of Andros Island on the west; it is here some 25 miles wide and it maintains this width for the greater part of its length as it stretches south. Between the southern extremity of Andros and Green Cay (see Chart A) it narrows to under 20 miles, but south of this point it expands eastward into an almost circular terminal basin of about 35 miles diameter. The total length is about 120 miles.

To the north, the Tongue of the Ocean is connected by a stretch of deep water, extending in a northeast direction, with the Providence NE. Channel and the Providence NW. Channel, leading respectively into the Atlantic and the Straits of Florida, and thus it is brought into direct connection with the two main divisions of the Gulf Stream.

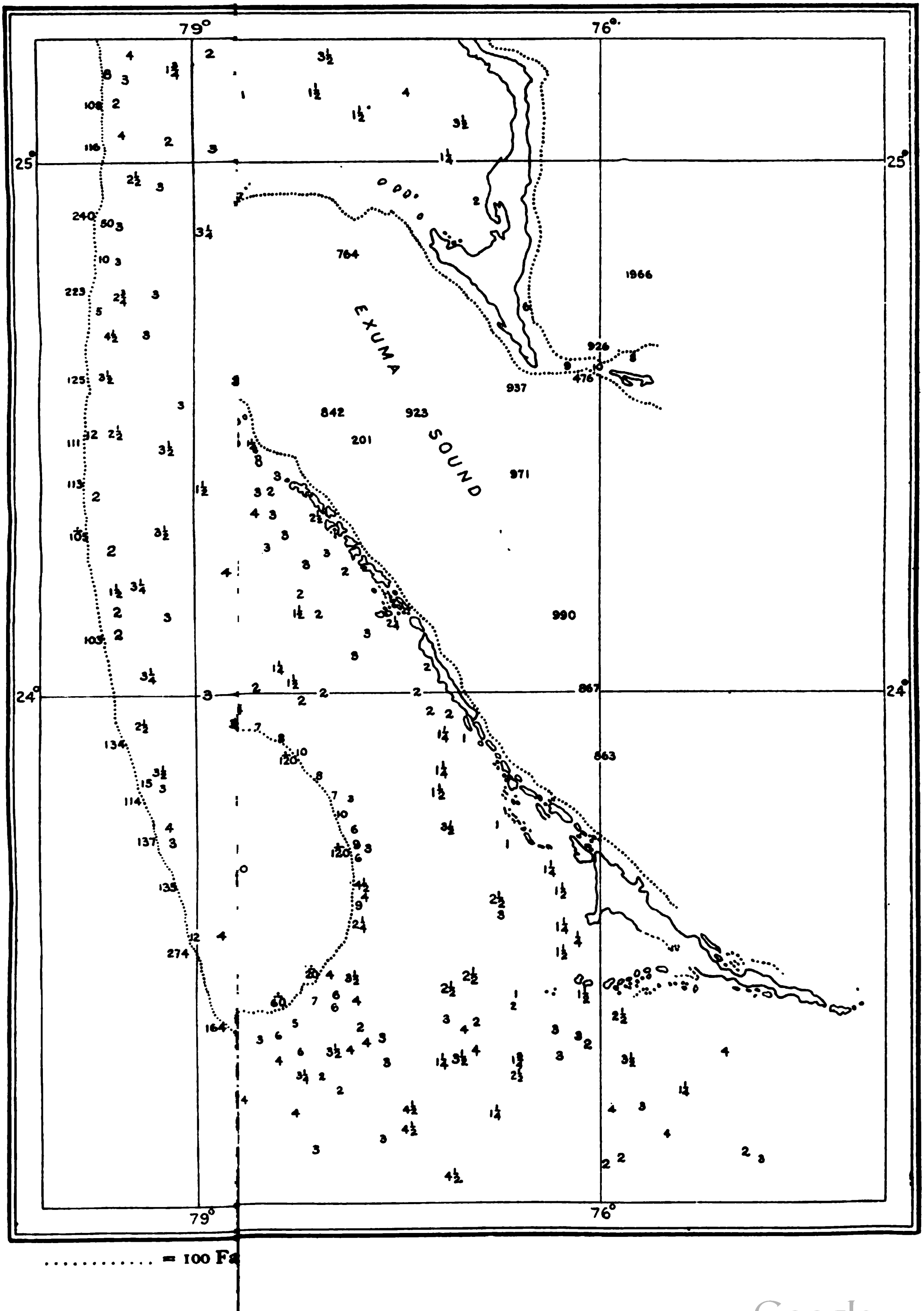
The soundings in this area are shown in Chart B. So far as observations go there seems to be a slight but regular upward gradient from a depth of 1,084 fathoms at the mouth to 740 fathoms in the southern terminal basin. Along the margins the gradient is extremely steep, and along the coast of Andros our observations showed that it was almost perpendicular at a distance of from one-fourth to one-half mile from the shore, but unfortunately no complete observations have been made from which this gradient could be calculated. When attempting to make soundings in this area, the sinker (a 30-pound conical iron weight) in every case was caught upon what was probably some projection on a submarine cliff, and it was impossible to free it; after several such attempts and loss of sinkers, the soundings were abandoned. The entrance to the Tongue by the Providence NE. Channel is of steadily increasing depth up to 2,200 fathoms at its junction with the still deeper water of the Atlantic; the Providence NW. Channel becomes shallower at its junction with the Straits of Florida, and between Great Isaac and the western end of Bahama Island is only between 200 and 300 fathoms in depth.

The surface water of the Tongue of the Ocean, except along the coast of Andros, is everywhere continuous with that overlying the thousands of square miles of shallows forming the Great Bahama Bank and the flats and cays lying to the north and west of the Exuma Islands, whereas the deeper water is only connected with the outer ocean by the comparatively narrow entrance between New Providence and the north of Andros, leading after a turn of about 90° into the Providence NE. and NW. Channels.

The laboratory from which this work was done was established at Golding Cay, at the eastern mouth of the South Bight of Andros; this position was especially suitable for the work, as by running out a few miles in a direction at right angles to the coast line the middle of the Tongue of the Ocean could soon be reached, and also the mud flats to the west of the island were readily accessible by water, as the South Bight runs right through the island to the west coast. In this region the tides are not strong, the average rise and fall being from 2 to 3 feet.

Much difficulty was experienced in getting any definite information as to the set of currents in the Tongue. Our local pilot stated that a current would set in a southerly direction for weeks at a time, and then without any apparent reason or change of wind would reverse and set in a northerly direction for several weeks; but such information derived from the negro natives can not be relied on. When taking observations on May 8, May 11, and May 23, we experienced a distinct southerly drift on each occasion,

CHART B



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but the amount of this drift was not determined, and moreover the drift caused by the wind was an unknown factor. On May 8 the wind was SSE., of force 1 at 8^h30^m a.m. freshening to force 3 at 10^h30^m a.m. A rough estimate from the landfall on returning gave the drift of the boat as about 2 miles south during the 4 hours occupied in working the station; the boat had a large awning and exposed a considerable area to the wind and had drifted this distance against the wind, so it would seem that on that occasion there must have been a strong current setting south.

Andros Island consists of a limestone formation, the exact nature of which has been dealt with by Dr. T. Wayland Vaughan (17). The greater part of the island is very flat and is elevated only a few feet above sea-level; a few irregular undulations, probably not more than 100 feet high, are found, especially along the east coast. There is evidence to show that formerly the level of the land was much higher than at present, and signs of rapid erosion of the rock are everywhere obvious. One of the most remarkable features is the absence of soil, even in the well-wooded parts of the island, the trees and bushes growing directly out of crevices and holes in the rock and giving rise to practically no leaf-mold. In the numerous "pot-holes" which occur all over the island, a small deposit of black leaf-mold can be found, and these "pot-holes" are the favorite places for the cultivation of sugar cane and bananas. The erosive action of water on the rock is especially noticeable where the slow drainage from an inland swamp can be traced in its course to the sea; in such a locality the hard rock is eroded, honeycombed, and undermined to a most remarkable degree, even though the amount of drainage, except after the heaviest rains, can scarcely be more than a slow trickle. Erosion of the rock along the sea-coast, where it is exposed to the action of the sea-spray, is also very marked. From the occurrence of this erosion it is obvious that all the water draining from the land into the sea must contain a high proportion of calcium salts in solution.

Towards the west of the island the land is remarkably flat, and near the coast consists of white chalky mud which has partially dried and in places has formed a harder crust on the surface. These half-dried mud flats slope almost imperceptibly into the sea and are continuous with the submarine flats which extend some 60 miles off the west coast with an average depth of 2 to 3 fathoms. The mud forming these submerged flats is very soft, and near the coast it was easily possible to push a 12-foot sponge-pole down to its full length into it without touching any harder material. The surface layer of the mud for a depth of about 6 inches is of a creamy white color, but below that it is of a grayish tinge and has a slight odor of sulphureted hydrogen. Unfortunately there was no opportunity of obtaining information as to the real thickness of this layer of mud, nor of investigating more than the surface layers at any distance from the coast.

Microscopical examination showed that this mud was almost entirely composed of minute unorganized particles of calcium carbonate. Near the shore a good deal of organic matter was present, chiefly in the form of decaying mangrove roots. Farther out from the shore little organic matter was noticeable, but it was not possible to examine the deeper layers of the mud in these situations. The only organic matter that was seen consisted of the rootlets of a species of *Zostera*, found in occasional patches some miles off the coast.

BACTERIAL INVESTIGATIONS IN THE DEEP WATER OF THE TONGUE OF THE OCEAN.

Continued bad weather during the whole of our stay at Andros greatly added to the difficulties of this work, and on this account it was only found possible to work three stations. The last two were worked under the most disadvantageous conditions, the quick roll of the boat making the filling of the water-bottle with alcohol and the siphoning off of the sample under sterile conditions a matter of the greatest difficulty.

The first station worked was 6 miles due east of Golding Cay, the second 14 miles east of Golding Cay, and the third 10 miles ENE.¼E. of Golding Cay. The three stations were thus at the angles of a triangle which was nearly equilateral, the base being a little longer than the sides and running due east and west.

At the first station, worked on May 8, bottom was sounded at 822 fathoms. The sea was calm at first, with a SSE. swell, but became choppy later. The wind was SSE., force 0 to 1, at 8^h30^m a.m., freshening to about force 3 at 10^h30^m a.m. The sample of the bottom obtained by the snapper-rod was of a very stiff clay-like consistency, grayish white in color, and was composed of very minute unorganized particles of calcium carbonate containing a few pteropod and globigerina shells. The following temperatures, to which the necessary corrections have been applied, were recorded:

Depth.	Temperature.	Depth.	Temperature.
Fms.	°C.	*Fms.*	°C.
Surface	26.90	400	9.03 8.93 } average = 8.98
10	25.90		
50	25.14	600	4.78 4.62 } average = 4.70
100	22.00		
200	17.13	Bottom (822 fms.)	4.00 3.94 } average = 3.97

These samples, without previous dilution, were plated in peptone agar, 1 c.c. of the sample being used for each plate. The agar was cooled to just under 40° C. before plating. It is very necessary that this temperature should not be exceeded, as many marine bacteria are very sensitive to heat; the use of agar at as high a temperature as 45° C. will cause the death of a large proportion of the bacteria, though in the process of plating they can be exposed to this temperature only for a very short time. The cultures were kept in the dark at the room temperature (averaging about 28° C.) and at the end of 24 hours a free growth of colonies was apparent. At the end of 48 hours the plates were counted with the following results:

Depth (in fathoms).	No. of colonies developing from 1 c.c. of sample, and remarks.
822 (bottom)	0
822 (bottom)	3
822 (bottom)	1
600	17
600	14
400	15
400	16
200	1,760 } Very much overcrowded; indications of presence of many more colonies undeveloped from overcrowding.
200	1,500
100	Uncountable owing to overcrowding.
100	Do.
50	Do.
50	Do.
10	Do.
10	Do.
Surface	Do.
Do	Do.

From these counts it is apparent that the number of bacteria falls off at some point between 200 and 400 fathoms.

The second station was worked on May 11 at a point 14 miles due east of Golding Cay. The sea was calm at first, and the wind ENE., force 1, but later in the day a heavy swell set in and the wind freshened to about force 4; eventually the weather became so bad that it was impossible to work, and the station had to be abandoned before it was completed. Bottom was sounded at 890 fathoms, but there was some stray on the wire, so that the true depth was probably about 825 fathoms, as shown by the chart. The bottom consisted of fine white calcareous ooze; no remains of pteropods were seen, but some globigerina shells were present. The following temperatures were recorded:

Depth.	Temperature.	Depth.	Temperature.
Fms.	°C.	*Fms.*	°C.
Surface	26.30	300	14.37 14.27 } average = 14.32
10	26.40	400	9.86 9.73 } average = 9.79
50	24.89	Bottom	4.17 4.13 } average = 4.15
100	22.63		
200	17.42		

Samples down to a depth of 200 fathoms were diluted 1 in 100 with sterilized sea-water before plating. The following results were obtained after 48 hours' growth:

Depth.	No. of colonies developing from 1 c.c. of sample.
Fms.	
Surface	16,200
10	13,100
50	14,000
100	14,000
200	15,000
300	14
400	12

The figures given represent the mean of the number of colonies developing in the two plates that were made from each sample. It is here apparent that the number of bacteria per 1 c.c. falls off very rapidly between 200 and 300 fathoms.

The third station was worked on May 23 at a point 10 miles ENE.½E. of Golding Cay. The wind was east, of about force 4. As it had been blowing for the previous ten days without intermission, the sea was so rough that it was only possible to work when steaming slowly ahead into the wind. This resulted in the production of a great deal of stray on the sounding-wire, so that the number of fathoms of wire run out is greater than the actual depth at which the samples were taken; these differences will be large for the more superficial samples, but small at greater depths, as the wire strays in a curve whose gradient becomes very steep a little below the surface under these conditions.

The following temperatures were recorded:

Length of wire run out.	Temperature.	Length of wire run out.	Temperature.
Fms.	°C.	*Fms.*	°C.
Surface	27.10	250	14.97 15.00 average=14.98
20	26.50		
100	22.80	350	10.85 10.84 average=10.85
160	18.83		

At this point the station had to be abandoned, owing to the bad weather. The samples down to 160 fathoms were diluted 1 in 100 with sterilized sea-water before plating in peptone agar; the remaining two were plated undiluted. At the end of 48 hours the following counts were made, representing the mean of the number of colonies in the two plates made from each sample:

Length of wire run out.	No. of colonies developing from 1 c.c. of sample.
Fms.	
Surface	15,000
20	15,500
100	13,700
160	13.300
250	14,300
350	16

The colonies developing in all the cultures were only of two kinds, the *Bacterium calcis* and the non-denitrifying species already described. The non-denitrifying species formed a relatively small proportion of the total, and were not found at all in cultures made from samples taken below 250 fathoms. As they appear to be comparatively inactive chemically, and as nothing is at present known concerning the part played by them in the metabolism of the sea, they will not be further considered here.

A consideration of these results obtained in the Tongue of the Ocean shows that the waters down to a depth of somewhere about 300 fathoms in April 1912 contained an enormously larger number of bacteria than the water in the neighborhood of Tortugas in June 1911. The number of bacteria falls off from about 14,000 to about 12 per 1 c.c. between depths of 250 and 350 fathoms; the temperature at 250 fathoms was about 15° C. and at 350 fathoms about 11° C., and it was shown in June 1911 at Tortugas that *B. calcis* will grow slowly at 15° C., but that growth is totally inhibited at 10° C. It would thus seem that the observed distribution of the bacteria agrees fairly with what might be expected from the temperature conditions.

As regards these observations on the occurrence of bacteria in small numbers at depths below 350 fathoms, the possibility of experimental error must be considered; a leakage into the water-bottle of 0.25 c.c., as it was being hauled up through the last 300 fathoms, would account for the number found, and there are also many possible sources of error in the process of siphoning off the sample and making the cultures where a permanent laboratory is not available. It is possible that the water below 350 fathoms was really sterile, though if so the constancy of the results obtained is curious if ascribed to experimental error. In any case, the small number of bacteria found at depths below 350 fathoms can play no part in the metabolism of the sea, since it has been shown that *B. calcis* is incapable of growth at the temperatures obtaining at these depths.

The much greater abundance of bacteria in the surface waters of the Tongue of the Ocean than in the waters around Tortugas may perhaps be accounted for by the fact that in the Tongue of the Ocean by far the greater part of the surface water must flow over the immense chalky mud flats and shallows which bound it in most directions and, as will presently be shown, these mud flats are phenomenally rich in bacteria and are probably still being deposited by bacterial agency.

HYDROGRAPHIC OBSERVATIONS IN THE TONGUE OF THE OCEAN.

The samples of water taken for hydrographic investigations were analyzed by Mr. D. J. Matthews at Plymouth. With great kindness he calculated the results, and from his notes the following observations and conclusions are drawn.

The samples were analyzed for salinity in comparison with the standard sea-water supplied by the Central Laboratory of the Conseil International pour l'Exploration de la Mer, and hence the results are strictly comparable with all other analyses published under the auspices of this International Council. The following results were obtained:

Depth.	At first station, 6 miles east of Golding Cay.				
	Temp.	Cl ‰	S ‰	σ_0	σ_t
Fms.	°*C.*	*P.ct.*	*P.ct.*		
0	26.90	20.06	36.24	29.12	23.70
10	25.90	20.46	36.96	29.71	24.57
50	25.14	20.43	36.91	29.66	24.76
100	22.00	20.28	36.64	29.45	25.48
200	17.13	20.08	36.27	29.15	26.48
400	8.98	19.58	35.37	28.43	27.43
600	4.70	19.49	35.21	28.30	27.90
822	3.97	19.367	34.98	28.11	27.79

Note.—The original surface sample was lost, owing to breakage of the bottle in transit to England. The analysis was made on a sample taken 3 days later at the same spot.

In this table Cl ‰ means the weight of chlorine in grams found in 1,000 grams of sea-water. S ‰ means the salinity, or total weight of salt in grams found in 1,000 grams of sea-water. σ_0 represents the specific gravity of the sample at 0° C., and σ_t represents the specific gravity of the sample at the temperature t at which it was collected, with no correction for pressure.

At the second station, 13.5 miles east of Golding Cay, the following results were obtained:

Depth.	Temp.	Cl ‰	S ‰	σ_0	σ_t
Fms.	°*C.*	*P.ct.*	*P.ct.*		
0	26.30	20.25	36.58	29.40	24.15
10	26.40	20.33	36.73	29.52	24.24
50	24.89	20.395	36.84	29.61	24.78
100	22.63	20.36	36.78	29.56	25.42
200	17.42	20.12	36.35	29.21	26.47
300	14.32	19.81	35.79	28.76	26.75
400	9.79	19.56	35.34	28.40	27.27
890	4.15	(No sample, bottle did not work.)			

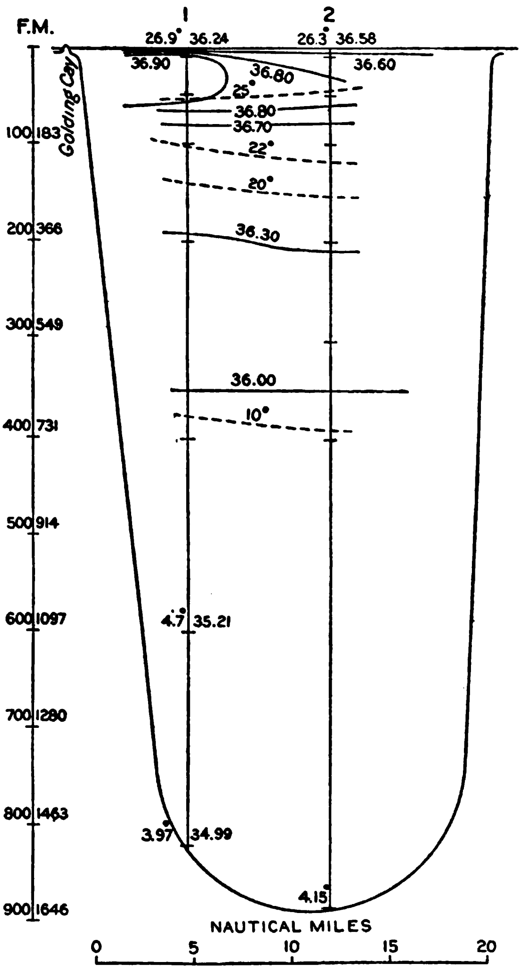

FIG. 2.—Section across the Tongue of the Ocean from Golding Cay, Andros Island, showing salinities and temperatures.

Owing to the uncertainty of the depths at the third station, due to the bad weather and consequent stray on the wire, it was decided not to include these observations in a consideration of the hydrographic conditions, and to make what deductions were possible from the results obtained at the two stations given.

Figure 2 shows the vertical distribution of layers of different salinities at the two stations in diagrammatic form.

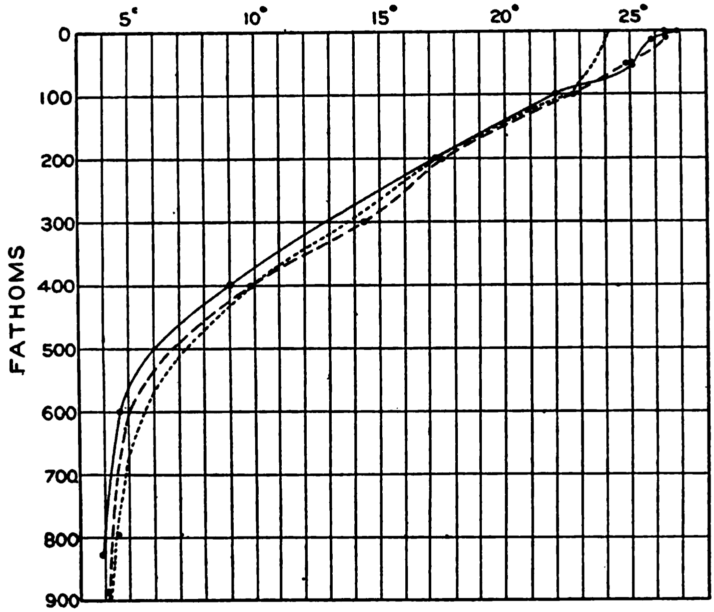

FIG. 3.—Temperature curves.
Continuous line = Station 1.
Broken line = Station 2.
Dotted line = *Challenger* Station No. 27.

The curves in figure 3 show the vertical distribution of temperatures at the two stations, and also give the temperatures obtained by *Challenger* Station No. 27 in 22° 49′ N., 65° 19′ W. March 28, 1873, where the depth was 2,960 fathoms. It is interesting to note that the actual reading at 200 fathoms at the latter station, 17.22° C., agrees more closely with the temperatures in the Tongue of the Ocean than that taken from the smoothed curve, which was 18.17° C.

The curves in figure 4 show the vertical distribution of salinities at the two stations, and also the salinities obtained by the *Michael Sars* in 37° 12′ N., 48° 30′ W., on June 25, 1910.

From these tables and diagrams it can be seen that the surface salinity increases from west to east very rapidly, 0.34 per cent in 7.5 miles, but the surface temperature is fairly uniform, between 26° C. and 27° C.

At both stations the salinity increases downwards to a maximum probably lying between 10 fathoms and 50 fathoms, but more rapidly at Station I, so that from 10 fathoms to 50 fathoms the salinity decreases from west to east.

Below 100 fathoms the conditions are closely similar at both stations, as far as the observations go; the salinity decreases fairly rapidly to 400 fathoms and then more slowly to the bottom.

The temperatures decrease rapidly and uniformly from the surface to about 500 fathoms, then more slowly to the bottom.

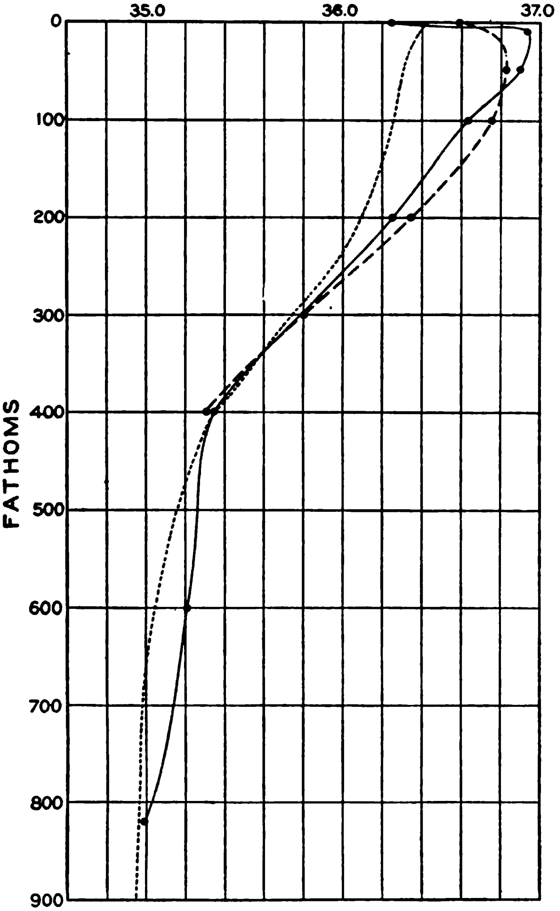

Fig. 4.—Salinity curves.
Continuous line = Station 1.
Broken line = Station 2.
Dotted line = *Michael Sars* Station No. 65.

There is practically no thermocline (German "sprungschicht") in the temperature at any depth and only a poorly marked one, confined to the upper stratum, in salinity.

The absence of a temperature thermocline is remarkable (see the curves for the two stations, and the two from the *Michael Sars* and the German South Pole Expedition, drawn for comparison).

In general, below about 250 fathoms the temperatures and salinities agree with the nearest stations of the *Michael Sars* in the open Atlantic;

above this depth they are higher, and differ from them and the open ocean north of the belt of calms, in the absence of a temperature thermocline, and in the maximum salinity being found below the surface. The latter points either to a considerable local supply of fresh water or to a current of lower salinity from either the Florida stream or the region of equatorial calms. Unfortunately we have no reliable salinity observations for the two latter.

With regard to the accuracy of the work, Mr. Matthews makes the following remarks:

THE ACCURACY OF THE OBSERVATIONS.

a. *Salinity.*—The method of taking the samples from the water-bottle was rather inconvenient, as a siphon was used; the samples were very small, but well preserved. The water-bottle itself might have leaked or closed at the wrong depth, as was the case with earlier models.[1] That this was not so is shown by

(1) The sharp fall in the number of bacteria at between 200 and 300 fathoms.

(2) The close agreement of the salinities at 400 fathoms, the greatest depth at which they were taken on both stations. Station I gave 35.37; Station II, 35.34.

(3) The close agreement between the bottom salinity at Station I, 34.98 at 822 fathoms, and the salinity found at the same depth at the nearest position at which we have modern observations—i. e., *Michael Sars* Station 65, in 37° 12′ N., 48° 30′ W., June 1910; according to the curve this is about 34.96.

b. *Temperatures.*—The National Physical Laboratory correction was given to 0.1° only, but the readings below 15° are comparable among themselves to 0.05° or possibly less. The curves of temperature for the two stations agree well in shape below 300 fathoms, but the temperature on Station II is generally slightly higher than on Station I, as a rule by an amount corresponding to a difference of depth of about 20 to 25 fathoms.

Below 200 fathoms the curves for both stations agree very closely with that for *Michael Sars* Station 64, in 34° 44′ N., 47° 52′ W.

It is almost certain from the above considerations that the results are only incorrect by the experimental errors in measuring the depth, in determining the salinity (0.01° at most), and by perhaps 0.1° C. of temperature.

These observations are sufficient to show that the Tongue of the Ocean is an area of considerable interest from a hydrographic point of view, and it is much to be regretted that the continued bad weather during our stay made it impossible to obtain more observations and samples.

BACTERIAL INVESTIGATION OF THE CHALKY MUD FLATS WHICH ARE BEING DEPOSITED TO THE WEST OF ANDROS ISLAND.

Samples of the mud were taken from the western entrance of South Bight (see Chart B), and from points 2 and 3 miles out from the shore; practically identical results were obtained from all these localities. The sample at the mouth of the bight was taken in about 4 feet of water, that 2 miles out in 7 feet, and that 3 miles out in 8 feet. The samples were necessarily taken from the surface of the mud.

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[1] The water-bottle only failed once, at about 890 fathoms at Station II.

For bacterial examination, one part of this mud was shaken up with three parts of sterilized sea-water; this was allowed to settle for 15 minutes, and then the clearer surface layer was diluted 1 in 1,000,000 with sterilized sea-water. The diluted fluid was plated in peptone agar, 1 c.c. being used for each of the plates. The count of a number of plates after 48 hours gave 40 colonies as an average, and thus the mud itself must contain 40 × 4 × 1,000,000 = 160,000,000 bacteria per 1 c.c. The actual number in the mud possibly exceeds this figure, since a large proportion of the bacteria would probably settle with the larger particles after the first dilution.

The bacteria found in these cultures were nearly all the *B. calcis;* only occasionally were a few colonies of the non-denitrifying species seen.

A sample of the water taken from the surface at a spot 3 miles out from the western entrance of South Bight gave a count of 35,000 colonies per 1 c.c., the great majority of these being *B. calcis*.

Subcultures of *B. calcis* were made in Gran's medium, and in the calcium succinate, calcium acetate, and peptone calcium acetate media, whose composition has already been given. Denitrification in all these media was rapid and eventually complete, and was accompanied by the precipitation of calcium carbonate. In the last three media, which contained no solid matter and were quite clear and transparent before inoculation, this precipitation was manifested after 12 hours by the formation of a thick white cloud in the fluid, readily distinguishable from the cloudiness produced merely by bacterial growth. The development of this precipitate continued rapidly during the first 48 hours, but in many cases it was composed of such fine particles that they showed little tendency to settle to the bottom of the flask; in other cases larger particles were formed and a sediment similar in appearance to the chalky mud of the mud flats was produced. The exact conditions determining the size of the particles precipitated could not be ascertained, as the size varied largely in cultures made at the same time, in the same media, and kept apparently under the same conditions. The addition of magnesium tartrate in small quantities (0.2 gram per 1,000 c.c.) to the culture media seemed to induce the precipitation of larger particles, but it did not appreciably affect the rate of growth of the bacteria. In some of the older cultures that had been kept for a week or more, the sides of the flasks were coated with a thin layer consisting of extremely minute rhombohedral crystals of calcium carbonate. Occasionally these crystals formed around small bubbles that had remained near the surface of the fluid, the weight of the crystals eventually caused the bubbles to sink, and then the contained gas became dissolved; in this way a number of small, hollow spheres were formed, their walls consisting of minute crystals of calcium carbonate. The formation of these curious bodies occurred especially readily in the calcium succinate medium to which 0.2 gm. of magnesium tartrate per liter had been added. The deposition of calcium carbonate in a distinctly crystalline form was only noted in old cultures, and then it was in an amount relatively extremely small when compared to the precipitate of unorganized and amorphous calcium carbonate.

Specimens of the precipitates from some of the culture media were sent to Dr. Fred. E. Wright, of the Geophysical Laboratory of the Carnegie Institution of Washington, who described them as follows:

Preparation I: Precipitate from medium composed of calcium acetate 5.0 grams, potassium nitrate 0.5 gram, peptone (Witte's) 0.2 gram, sea-water 1,000.0 c.c., filtered and sterilized, contains two substances: (1) Fine grains of a strongly birefracting, apparently uniaxial, optically negative substance and with refractive index about 1.66. This is probably calcite. The grains are isolated, and no evidence of spherulitic crystallization was observed. On treatment with very dilute hydrochloric acid a noticeable evolution of carbon dioxide took place. (2) Scattered through the preparation are fine needles of a weakly birefracting substance of refractive index of about 1.525; extinction angle large. These needles are evidently selenite (hydrated calcium sulphate).

Preparation II: Precipitate from medium composed of calcium succinate 2.0 grams, magnesium tartrate 0.2 gram, potassium nitrate 0.5 gram, sea-water 1,000.0 c.c., consists largely of a cryptocrystalline aggregate of a weakly birefracting substance, whose refractive index is about 1.52 to 1.53. This substance proved too fine for further determination. Scattered through this substance are rounded and irregular patches of a second cryptocrystalline substance of strong birefringence, which gives off CO_2 when treated with dilute hydrochloric acid, and is probably calcite.

Preparation III: Precipitate from a medium composed of calcium acetate 5.0 grams, sodium phosphate ($Na_2HPO_4 12H_2O$) 0.25 gram, potassium nitrate 0.5 gram, sea-water 1,000.0 c.c., is again very fine-grained and consists (1) in large measure of minute grains of a substance which agrees with calcite in its optical properties in so far as they could be determined. On immersion in dilute HCl a distinct evolution of CO_2 gas was observed. (2) Of a substance whose grains are somewhat coarser than the calcite grains, their birefringence being medium to weak; refractive index about 1.525; biaxial and apparently optically positive; probably selenite, but not crystallized in the usual manner.

The small quantity of hydrated calcium sulphate present in these precipitates is undoubtedly derived from that in solution in the sea-water with which the media were made up, but the reason of its precipitation is difficult to explain, since no such precipitation occurred in culture media kept uninoculated under similar conditions as control experiments. It would therefore appear that this deposition of calcium sulphate along with the calcium carbonate must in some indirect way be the result of bacterial action, and it would seem a possible suggestion that the odor of sulphureted hydrogen noticeable in the deeper layers of the mud flats might be due to the reduction of the calcium sulphate to a sulphide, and subsequent decomposition of the sulphide.

These observations have shown that on the chalky mud flats of the Great Bahama Bank the *B. calcis* is found in enormous numbers, and also that this bacterium is capable of precipitating calcium carbonate from fluid media containing soluble calcium salts. It would seem a fair deduction that these mud flats, consisting of fine unorganized particles of calcium carbonate, have been precipitated by the action of the *B. calcis* on the soluble calcium salts carried into the sea by drainage from the land, where extensive and rapid weathering of the limestone rock is in progress.

CONCLUSION.

The observations so far available are too few, and the area they cover too small, to attempt to make any broad generalization at present. However, it can be stated with a fair degree of certainty that the very extensive chalky mud flats forming the Great Bahama Bank and those which are found in places in the neighborhood of the Florida Keys are now being precipitated by the action of the *Bacterium calcis* on the calcium salts present in solution in sea-water. From this the suggestion is obvious that the *Bacterium calcis*, or other bacteria having a similar action, may have been an important factor in the formation of various chalk strata, in addition to the part played by the shells of foraminifera and other organisms in the formation of these rocks. Dr. T. Wayland Vaughan has also suggested that the Miami oolite and other oolitic rocks may owe their origin to the occurrence of some diagenic change in the precipitate of very finely divided particles of calcium carbonate produced in this way by bacterial action. If this view as to the formation of chalk and oolite rocks is correct, it would seem probable that these strata must have been deposited in comparatively shallow seas whose temperature approximated to that of tropical seas at the present time.

It has also been shown that bacterial denitrification is far more rapid and complete in the tropical seas around Jamaica, Tortugas, and Andros than in the temperate waters of the Bay of Biscay and the English Channel, and hence an explanation is provided of the relative scarcity of plankton and algal growth in the former localities, in accordance with the terms of Brandt's hypothesis.

The distribution of the bacteria, both as to numbers and species, has been shown to vary at different localities and at different depths, but there are at present too few observations to enable any conclusions or generalizations to be drawn.

As it now stands, the investigation can at most be considered to offer a mere indication of the part played by bacterial growth in the metabolism of the sea. To obtain a real insight into the question, it would be necessary to make more extensive bacterial and chemical observations in tropical, temperate, and arctic waters, to study the bacteriology of other areas where calcium carbonate is being precipitated from the sea, and to make further investigations in the laboratory into the chemistry of the reactions that can be brought about by various species of marine bacteria.

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III.

PRELIMINARY REMARKS ON THE GEOLOGY OF THE BAHAMAS, WITH SPECIAL REFERENCE TO THE ORIGIN OF THE BAHAMAN AND FLORIDIAN OOLITES.

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Geologist in charge of Coastal Plain Investigations, U. S. Geological Survey.

PRELIMINARY REMARKS ON THE GEOLOGY OF THE BAHAMAS, WITH SPECIAL REFERENCE TO THE ORIGIN OF THE BAHAMAN AND FLORIDIAN OOLITES.[1]

By Thomas Wayland Vaughan.

At Doctor Mayer's request the following preliminary statement on certain aspects of Bahaman geology and on the origin of the Bahaman and Floridian oolites is submitted as a companion paper to that of Mr. Drew, whose untimely death is both a source of great sorrow to his friends and a heavy loss to science. As Drew's investigations have proven an essential step in the solution of the problem of the origin of the extensive oolitic limestone formations of Florida and the Bahamas, it is fitting that an account of the processes by which they originate should accompany his posthumous memoir. Although the investigation of the rock specimens and bottom samples obtained in the Bahamas and Florida from April to July 1912 is not complete, certain definite and some tentative conclusions have been reached which may here be appropriately stated.

The Bahama Islands and their accompanying shoals occupy a triangular area which lies east of Florida and north of the islands of Cuba and Haiti. The northwest corner of the triangle is in latitude 27° 35′ N., longitude 79° 2′ W., about 40 miles north of Jupiter Inlet on the Florida coast. The southeast corner is Turks Islands, north of Haiti, in latitude 21° 20′ N. and longitude 71° 5′ W. The western side is a nearly north-and-south line from Little Bahama Bank to about latitude 25° 30′, whence the boundary bends into a south-of-east direction. The islands either occur on one of two large banks, the Little Bahama and the Great Bahama Banks, or they rise to the southeast of the latter bank as isolated eminences separated by water as much as 1,000 fathoms in depth.

The shoalest water in the bottom of New Providence Channel between Little and Great Bahama Banks is 253 fathoms in depth; that in the Straits of Florida, between the former bank and the Florida coast, off Jupiter Inlet, is 341 fathoms deep; between the latter bank and Florida the depth is 422 fathoms. The Great Bahama Bank is separated from Cuba by the Old Bahama Channel, which ranges from 276 to 296 fathoms in depth. This bank is indented by two bodies of deep water. The first of these, theTongue of the Ocean, is sack-shaped and lies east of Andros Island and west of the bank, on the northern end of which is New Providence Island. Its depth ranges from 795 to 1,200 fathoms. The second indentation, Exuma Sound, is eastward from the Tongue of the Ocean, across the bank and keys extending southward from New Providence Island. This sound is purse-shaped and exceeds 1,000 fathoms in depth. Water 1,000 fathoms in depth is close to the eastern shore of the Bahamas as far north as Elbow Cay on Little Bahama Bank. Eastward of the 1,000-fathom curve the bottom rapidly descends to a depth between 2,000 and 3,000 fathoms. The Bahama Islands are subaerial protuberants above the nearly level, slightly submerged surfaces of extensive plateaus which on one or more sides rise precipitously from oceanic depths.

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[1] The investigations, the results of which are here presented, were conducted under the joint auspices of the Department of Marine Biology of the Carnegie Institution of Washington and the United States Geological Survey. The former organization furnished opportunities for field work, while the latter authorized the prosecution of the investigation as a part of my official duties, and furnished facilities for laboratory studies in Washington.

The water from Gun Key to Northwest Passage, a distance of 67 knots, is only 7 to 12 feet in depth, while the bottom southeast of New Providence Island ranges from awash to 12 or 18 feet below water-level. New Providence Island is mostly a platform from sea-level to 20 feet in elevation, with several east and west ridges standing above it; the highest of these, Nassau Ridge, rises to an altitude of about 100 feet. Andros Island has a similar physiography, a nearly level platform ranging in height from sea-level to 20 feet above that datum plane. Along its eastern front, lying near the shore, is a series of interrupted hills that range in elevation from 20 to 60, and perhaps in some instances to 100, feet. West of the coastal hills the surface is low, usually less than 10 feet in elevation, while much of the areas along the west front is affected by the tides. The Barrier Reef of Andros stands on the seaward edge of a shallow, submarine platform, at a distance of one-half mile to 2 miles off shore. The slope of the bottom seaward of the reef is comparatively rapid; between the reef and the shore the depth ranges from 1 to 2 fathoms. The platform between the reef and the shore is composed of hard lime rock, the surface of which is indented by numerous submarine pits. The deepest of the holes, locally known as "blue holes," sounded was 36 feet deep. As the general aspect of these holes is similar to the abundant subaerial pot-holes, locally known as "banana holes," formed by solution of the limestone, they are evidence of subsidence.

The general country rock of the Bahamas is oolite. The sea bottom from Gun Key to Northwest Passage, New Providence Island, and Andros Island is all or mostly composed of oolite, precisely similar to that of southern Florida. The material forming the ridge at Nassau has been wind-blown, and evidently is a dune accumulation. Many or all of the ridges rising higher than 20 or 30 feet above sea-level have been formed of wind-blown material, of which oolite is an important constituent, but the oolite of most or all the lower areas is of marine origin and has not been subjected to æolian action.

The finely divided calcium carbonate oozes in the shoal waters of Florida and the Bahamas have long attracted attention. Louis Agassiz, as early as 1851, noted them, as did Captain Hunt, Alexander Agassiz, and others.

Dall, so far as I am aware, was the first to suggest that "much of the limy deposit of the area behind the reefs and defended by them is probably the result of the deposition of lime originally in solution and precipitated by chemical action rather than of mechanically transported sediment."[1] Later, Samuel Sanford became convinced that the flocculent oozes in some of the Florida bays and sounds were chemically precipitated, and so expressed himself.[2] The most careful study as yet made of the shoal-water bottom deposits of south Florida was the one initiated by me in 1908 and conducted in conjunction with George C. Matson.[3]

This investigation led to the conviction that the flocculent, so-called amorphous, calcium carbonate is neither of detrital nor of organic origin, and therefore must be a chemical precipitate. In 1911, G. H. Drew came to the Tortugas Laboratory for the purpose of studying denitrifying bacteria. As he discovered that ammonia was a product of the metabolism of these organisms he was led to conduct experiments to ascertain the effect they might have in precipitating calcium carbonate. They were found to be active in Florida; and investigations he subsequently made in the Bahamas showed 160,000,000 of these bacteria to the cubic centimeter of surface mud on the west side of Andros Island. Drew says:[4]

> A consideration of these observations shows firstly that *B. calcis* is found in enormous numbers in the chalky mud flats of the Great Bahama Bank, and secondly that this bacterium is capable of precipitating calcium carbonate from fluid media containing soluble calcium salts. It would seem a fair deduction that these mud flats have been precipitated by the action of *B. calcis* on the soluble calcium salts carried into the sea by drainage from the land, where extensive and rapid weathering of the limestone rock is in progress.

Drew has shown what we may be confident is the major agency in producing the precipitation of the calcium carbonate ooze.

Every geologist who has studied the Florida oolites, except Alexander Agassiz, has considered them marine deposits; and Louis Agassiz, Sanford, and myself considered them associated with or derived from the fine calcareous muds. My opinion[5] was more definitely expressed than those of the other investigators.

Rainey, as early as 1858, showed, in a remarkable book entitled "On the mode of formation of shells of animals, of bone, and of several other structures, by a process of molecular coalescence, demonstrated in certain artificially formed products," that calcium carbonate precipitated from water-soluble salts of calcium in a viscid solution form spherulites and not crystals. In 1871, Harting, in a memoir, "Recherches de morphologic synthétique sur la production artificielle de quelques formations calcaires organiques,"[6] showed that calcium carbonate precipitated from a solution of a water-soluble calcium salt by the addition of the carbonate of potassium or sodium is at first of gelatinous consistency and later aggregates into spherulites.

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[1] U. S. Geological Survey Bull. No. 84, p. 101. 1892.

[2] Second Annual Report Florida Geological Survey, p. 228. 1910.

[3] A contribution to the geologic history of the Floridian Plateau. Carnegie Institution of Washington Pub. No. 133, pp. 114–145. 1910.

[4] Carnegie Institution of Washington, Year Book No. 11, p. 144. 1912.

[5] Carnegie Institution of Washington Pub. No. 133, pp. 173–177. 1910.

[6] Verh. Kon. Akad. Wetensk., Amsterdam, vol. 13, pp. 84, 4 pls. 1871.

In 1903, G. Linck published, in an article entitled "Die Bildung der Oolithe und Rogensteine,"[1] accounts of a series of important experiments he conducted on the precipitation of calcium carbonate from sea-water by the addition of alkaline carbonates, and in 1909 another contribution from him, "Ueber die Bildung der Oolithe und Rogensteine," appeared in the Jenaische Zeitschrift für Wissenschaft, vol. 45, pp. 267–278. According to Linck's experiments calcium carbonate precipitated from sea-water at a temperature of 17° C. to 40° C., by adding an alkaline carbonate, is aragonite and aggregates into spherulites, the diameters of which range from 0.02 to 0.2 mm. Murray and Irvine have shown that calcium carbonate precipitated from sea-water by an alkaline carbonate at a temperature of 34° F. is calcite, at 47° F. is a mixture of calcite and aragonite, at 80° and over is aragonite.[2]

Drew in 1912 published the following statement regarding the calcium carbonate precipitated[3] in a culture from a sample of water from Marquesas Lagoon:

> To such a culture a trace of very finely powdered hydrated calcium sulphate was added. This resulted in the formation of a precipitate, which, on microscopical examination, could be seen to consist of finely laminated concretions, some of which appeared to have a particle of calcium sulphate or sand as a nucleus. The concretions were soluble in dilute hydrochloric acid with evolution of carbon dioxide. These concretions bear a resemblance to those of some oolitic limestones, and the experiment suggests the manner in which such oolites may have been formed.

These references, although they lay no claim to completeness, are clear evidence that calcium carbonate, particularly aragonite, when precipitated from a water-soluble calcium salt by the addition of an alkaline carbonate, is at first of gelatinous consistency and later aggregates into spherulites.[4]

As I did not observe oolite grains in the muds when collected in the field, I was led to the opinion that oolitization was the result of secondary changes after precipitation and directed my attention to ascertaining if such supposed changes were taking place.[5]

Bahaman shoal-water bottom muds were collected through South Bight and off the west shore of Andros; and other samples were collected in Florida. As has been stated, these flocculent oozes when collected were not observed to contain oolite grains; however, such grains may have been present and may have escaped attention. All the Bahaman muds when examined at the end of November did contain oolite. Besides oolite grains, finely divided particles less than 0.001 mm. in diameter, some fragmental calcium carbonate, alcyonarian spicules, shoal-water foraminifera, and other micro-organisms were present.[1]

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[1] Neues Jahrb. für Min. Beilage, Bd. 16, pp. 495–513.

[2] Proc. Roy. Sci. Edin., vol. 17, pp. 79–109. 1890.

[3] Carnegie Institution of Washington, Year Book No. 10, p. 141. 1911.

[4] Without making a special diversion to go into the subject, it may be mentioned that spherulites or oolites of calcium malate and calcium phosphate are well known, and that the occurrence of spherulites of barium carbonate and strontium carbonate have been recorded. It is therefore established that a number of salts of the calcium group of elements may assume a spherulitic form.

[5] Carnegie Institution of Washington, Year Book No. 11, pp. 155, 157, 158. 1913.

Dr. Fred. Eugene Wright has examined some of the bottom samples for me and reports that the particles large enough for ascertaining their optical properties are calcite, but the fine material can not be optically determined. A series of the muds and several powdered specimens of Pleistocene oolites from Florida and the Bahamas were tested with the cobalt nitrate reaction; and as all of these upon heating gave the purple color characteristic of aragonite, this form of calcium carbonate was shown also to be present. The muds and the Pleistocene oolites, therefore, are composed of a mixture of aragonite and calcite.

The grains of the Bahama and Florida oolites range in size from 0.10 to 0.80 mm., and occasionally a grain perhaps exceeds 1 mm. in diameter. The spherulites or spheroid aggregates in the muds range from 0.004 or 0.006 mm. to oolite grains of ordinary size.

In order to test the growth of the grains, samples of a number of muds were strained through No. 10 bolting-cloth, which has a mesh of about 0.13 mm. in diameter, and the fine material that passed through the bolting-cloth was put into bottles containing sea-water. These bottles were permitted to stand from about November 25 until the first week in March, over three months, when a small portion of each sample was again strained through No. 10 bolting-cloth, and the portion retained on the cloth studied. The formation of oolite grains was found to be in progress in every sample, and numerous grains had apparently grown to such a size as to preclude their passing through the mesh of bolting-cloth. The grains showed the usual forms of oolite grains: spheroids, ovoids, and ellipsoids. The larger grains had smaller diameters of 0.17 mm.; longer diameters up to 0.23 mm. Those newly formed are soft and easily crushed by any kind of pressure. The experiments indicate increase both in number and in size of grains.

The precipitated calcium carbonate may segregate around a variety of nuclei, for instance, spherulites or round aggregates formed of the precipitated material, small grains of sands, shells of foraminifera, and gas bubbles.[2]

Although there is need for additional study of the factors that accelerate, retard, or inhibit the formation of spherulites and initial round aggregates and the subsequent growth of the grains, the empirical facts in the process of the formation of the Floridian and Bahaman oolites are clear. They are as follows:

1. Denitrifying bacteria are very active in the shoal waters of both regions and are precipitating enormous quantities of calcium carbonate which is largely aragonite.

2. This chemically precipitated calcium carbonate may form spherulites or small balls which by accretion may become oolite grains of the usual size, or it may accumulate around a variety of nuclei to build such grains.

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[1] The investigation of these muds is still in progress [June 1913] and will not be finished for some months, The completed results will appear in a subsequent publication.

[2] See the papers by Linck, Drew, and Vaughan, already cited.

The deduction may be made that all marine oolites, originally composed of calcium carbonate, of whatever geologic age, may confidently be attributed to this process.

Two other important deductions may be made from the knowledge of this process, viz:

1. Neither the Bahamas nor the oolitic keys of southern Florida are coral islands, but they have been formed by this other process. Elevated coral rock is exceedingly scarce in the Bahamas and the Recent reef of Andros is comparatively insignificant as a constructional geologic agent. The material composing the land masses and much or most of the submarine platforms of the Bahamas is thus removed from the category of "coral rock" and the living reef reduced to a subordinate ratio as a builder of limestone.

2. Drew's studies (unfortunately incomplete) of the distribution of denitrifying bacteria have shown them to be most prevalent in the shoal waters of the tropics. They therefore conform to the temperature relations enunciated by Murray[1] for the distribution of lime-secreting organisms. By combining the results of Drew and Murray, the deduction seems warranted that great limestone formations, whether they be composed of organic or of chemically precipitated calcium carbonate, were laid down in waters of which at least the surface temperatures were warm, if not actually tropical.

This brief paper may be closed with the following statement and tentative outline of the geologic history of the Bahamas, with special reference to the eastern part of Andros Island:

The presence of oolite containing Pleistocene marine fossils above sea-level indicates elevation in the Bahamas; the presence of submerged solution pot-holes, not filled with sediment, indicates that the last movement was downward.

TENTATIVE OUTLINE OF THE PLEISTOCENE AND RECENT GEOLOGIC HISTORY OF THE BAHAMAS, AND ESPECIALLY OF ANDROS ISLAND.

(1) During Pleistocene, perhaps earlier Pleistocene time, numerous shallow submarine banks existed, on which calcium carbonate was chemically precipitated mostly as aragonite, and this was converted into oolite.

(2) This submergence was followed by uplift with the production by marine erosion of a platform and a margining shore cliff, and the formation of shore dunes paralleling the windward sea-front.

(3) Apparently there was further uplift and pot-holes were subaerially formed by solution on the platform between the dunes and the sea-front. At this time the land on the east side of Andros Island stood about 40 feet higher than now.

(4) The last event was a subsidence of about 40 feet, admitting the sea back to the edge of the previously cut low scarp, and the present barrier reef developed on the off-shore margin of the submerged platform.

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[1] Natural Science, vol. 2, pp. 25–27. 1897.

IV.

THE BUILDING OF THE MARQUESAS AND TORTUGAS ATOLLS AND A SKETCH OF THE GEOLOGIC HISTORY OF THE FLORIDA REEF TRACT.

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IV.

THE BUILDING OF THE MARQUESAS AND TORTUGAS ATOLLS AND A SKETCH OF THE GEOLOGIC HISTORY OF THE FLORIDA REEF TRACT.

BY THOMAS WAYLAND VAUGHAN.

INTRODUCTION.

The following brief article is supplementary to my paper "A contribution to the geologic history of the Floridian Plateau,"[1] in which it is stated that "the geologic history of the Marquesas and Tortugas is reserved for future consideration." The study of the geology and the geologic processes of the Florida reef tract, and especially of the Tortugas and the Marquesas, has been continued since 1910. During the field season of 1913 Mr. R. B. Dole, of the U. S. Geological Survey, undertook a special investigation of the solvent effect, by virtue of the content of carbon dioxide, of sea-water flowing into and out of Tortugas Lagoon. As the various lines of investigation have given definite results, the principal factors in the formation of the Marquesas and Tortugas atolls and their inclosed lagoons now seem clear, although some details still require elucidation. It is now also possible to outline with a feeling of confidence the salient geologic episodes in the history of the entire Florida reef tract and to institute comparisons with other coral-reef areas. As this paper is only a preliminary statement, it is intended subsequently to publish a more comprehensive discussion of the different problems here treated in a cursory manner.

BUILDING OF THE MARQUESAS AND TORTUGAS ATOLLS.

An inspection of the charts of the U. S. Coast and Geodetic Survey, No. 1252 for the Marquesas and No. 471*a* for the Tortugas, will show a striking similarity in the configuration of both, for in each banks or keys surround a central depression and in each there are major entrances in the southeast, southwest, and northwest quadrants. The Tortugas, however, are of a more elliptical form than the Marquesas, the axis of the ellipse extending from northeast to southwest. The Marquesas lagoon is only 1 to 15 feet in depth, while that of the Tortugas is from 7 to 13 fathoms. In both the principal key or bank slightly under water extends from the southeast to the northwest entrance, or is on the east, northeast, and north sides. The keys and banks between the southeast and southwest passages and between the southwest and northwest passages are minor as compared with the larger one mentioned. It may also be pointed out that the Marquesas occur just west of Boca Grande Channel, and that the Tortugas lie just west of Rebecca Channel.

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[1] Carnegie Institution of Washington, Pub. 133, pp. 99–185, 15 pls. 1910.

That the Marquesas were formed through the agency of waves and currents operating under proper conditions has been evident for some time, but the processes by which the Tortugas were built were less clear. It was thought possible that submarine solution had been efficacious in the latter, while it was apparent that it was ineffective in the former. In order to answer the question for the Tortugas, two lines of inquiry were undertaken. One of these was the examination of the bottom of the Tortugas Lagoon, to discover whether or not sediment is there accumulating; the other was to ascertain by chemical examination whether or not there was any excess of carbon dioxide in the water flowing into the lagoon and whether there was any difference in the quantity of carbonates in the outflowing and inflowing water. The latter investigation was undertaken by Mr. R. B. Dole. The bottom samples collected at numerous localities in the Tortugas established that the bottom in the channel between Garden Key and Bush Key, in Bird Key Harbor, and in the lagoon channels west and east of White Shoal and north of that shoal is formed of soft calcareous mud, largely of the kind Drew showed to be precipitated by denitrifying bacteria. The examination showed conclusively that sedimentation predominates over the removal of material, perhaps except where currents may corrade the sides or bottoms of the channels along which they flow. The conditions in the Tortugas lagoon are similar to those in the bays and sounds behind the Florida keys and to those in Marquesas Lagoon, in all of which filling by deposition is progressing.

The specimens examined by Mr. Dole were collected twice a day on the principal flowing and ebbing tides for nearly a lunar period, from May 20 to June 15, near buoy No. C3, in Southwest Channel, off the southern end of White Shoal. Mr. Dole's results are summarized on page 74 of this publication. They show that none of the water, neither that flowing into nor that flowing out of the lagoon, contains any dissolved free carbon dioxide, that all the carbon dioxide in the water is present in the carbonate or bicarbonate form, and that the inflowing water possesses no power to dissolve calcium carbonate by virtue of its content of carbon dioxide, as its capacity for the solution of lime has been exhausted before flowing into the lagoon. The chemical investigations of the waters and the study of the bottom deposits gave mutually confirmatory results, that the water flowing through the Tortugas Lagoon does not dissolve calcium carbonate and that the lagoon has not been formed by solution.

As two kinds of phenomena observed in the Tortugas might be considered to afford evidence in favor of solution, both will be briefly discussed.

One is the frequent occurrence of submerged coral heads, the interiors of which have been removed, leaving a partially inclosing skeletal shell covered by living polyps. Some of these heads have a suggestive resemblance to atolls, but the resemblance is only superficial. The excavation of the interior is to be explained by the upper surface of the envelope of living tissue having been damaged in some way, thus giving an opportunity for boring organisms of great variety to begin and prosecute their work of disintegrating the skeleton. Waves and currents wash out the broken-up material, forming a cavity which is bounded by a shell of substance whose outer surface is covered by living polyps. The other phenomena that might be attributed to solution are the overhanging projections along the steep, submarine cliffs or rock pinnacles which in places occur on the sides of channels. Since these stand against the sides of the currents it is probable that submarine scour, corrasion, has been a factor in producing them; and subaerial modification deserves consideration, as will later appear. As the dynamic agencies have been more and more thoroughly studied, the necessity for referring any phenomena observed in the Tortugas to submarine solution has been eliminated, and the conclusion definitely reached that such solution produces no geologic effects of importance.

As the production of the Marquesas and Tortugas atolls with their inclosed lagoons by solution is eliminated, other factors, especially waves and currents, will be considered. There are two current-shaped physiographic forms to which it is desired particularly to call attention—these are current-shaped crescents and current-shaped linear ridges. With reference to crescents it may be said that if an obstruction lies across the line of direction of a constant current, the current shears to each side and will drift detrital material in such directions as to form a crescentic accumulation, the bow of the crescent facing the current, while the horns of the crescent will curve before the current. An eddy may be produced in the space between the horns of the crescent, and a deposit may be formed there.[1]

Crescentic sand-dunes and crescentic keys are both well known. Should there be no cross obstruction, currents both aerial and aqueous will form linear ridges, under conditions which induce dropping material. Both linear sand-dunes and linear submarine banks are well known. Of current-transported detritus, crescentic accumulations lie across the main direction of the current, while linear ridges lie along its direction.

The currents of the Florida keys are of three kinds: wind-formed currents, which are accompanied by waves; the Florida counter current; and tidal currents. The winds at Key West, where accurate records have been kept, prevailingly range in direction from northeast to southeast, those from the east predominating over those from any other direction. Both the Marquesas and the Tortugas lie within the tract of the Florida counter current, which moves in general toward the west. The directions of the wind-formed currents and the counter current are concordant and they therefore cooperate. The tidal impulse, however, is roughly from north to south and the direction of tidal currents is therefore transverse to the other currents.

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[1] Dr. A. G. Mayer called my attention to the occurrence of this eddy.

The principal arcs of the rims of both the Marquesas and the Tortugas, *i. e.*, those from the southeast to the northwest entrance, are bowed toward the east, against the prevailing direction of the wind and that of the counter current, while the northern limb of the arc in each instance trails with these currents. These arcs therefore satisfy the requirements of wave and current-shaped arcs.

The southern sides of both the Marquesas and Tortugas are broken by southeast and southwest passages. These breaks are probably to be attributed to cross-tidal currents. Also both the Marquesas and Tortugas have a northwest passage. The explanation of this is probably as follows: In the Tortugas, although the data on currents are inexact, the available evidence indicates a stronger tidal inflow than outflow, much of the water of the receding tides not passing through the lagoon but around the outside of its rim. The water coming into the lagoon would tend to be driven toward the west by the winds, and would consequently find its outlet in the northwest quadrant.

In the Marquesas, the land area west of the southeast passage follows a line that would be expected of the southeast horn of a crescent formed under conditions of wind and currents known to prevail there. The formation of the bank and the small keys on it on the west side of the lagoon is more obscure. Perhaps they were formed by winds and accompanying waves that come, especially during hurricanes, from the west and by a current eddy produced to the leeward of the bow of the crescent.

The main agencies whereby the principal arc of the rim of the Tortugas was formed seem definitely known, but those building other parts of the rim are still somewhat vague. Between what is known as Five-foot Channel and Southwest Passage are holes, some of which are 6 fathoms or more in depth. It is evident from the distribution of these holes that once there were several channels trending more or less from southwest to northeast and approximately following the course of tidal flow, the channels bounded by banks elongate in the same direction. Detritus carried westward by waves and currents has filled parts of the channels and broken them up into holes, some series of which may still be recognized.

Loggerhead Key, as well as the bank on which it stands, is elongate from southwest to northeast and has each of its ends somewhat curved toward the west. The general direction of the bank is approximately along that of tidal flow, but it is not an unmodified linear ridge coinciding in direction with that of one current, for it is evident that the prevailing run of the waves before the wind, and perhaps the counter current acting at its southern end, have curved its ends westward. The effect of waves running before the wind in shaping a key was studied in detail on Loggerhead Key, where both the north and south horns of the key curve before the prevailing easterly winds. A reversal of a part of the horn of the north end of the key was observed during a swell from the northwest. During this reversal of the direction of the run of the waves, a reversed crescent was formed at the end of the spit, the bow of the crescent being toward the current while the horns curved before it. The more indurated beach rock on each side of Loggerhead Key slopes toward the sea, showing that the key is wave-built, on the surface at least. In this connection it may be said that a current too weak to pick up and carry detritus will, when agitation has lifted particles above the bottom, appreciably move them in the direction of the current. Loggerhead Key stands on the lee side of the lagoon, where waves from the east would be weaker than on the windward side, and where waves from the west would exert their full force. The following is offered as an hypothesis for the formation of the bank on which this key stands: It is largely or mostly a detrital ridge, formed in a partial eddy on the leeward side of the lagoon, mainly along the direction of flow of the stronger tidal currents. Wave agitation from the east and west has facilitated the movements of detritus and has built the sloping beach rock on both the lagoon and the sea sides. The stronger winds from the east have given a slight westward curvature to the extremities of the bank.

Before leaving the discussion of Loggerhead Bank and Key, attention will be directed to changes that have occurred since October 1910, when the last severe hurricane visited this region. Since that time the waves have acted with only slight cessation on the eastern side of the key and have cut away the eastern side of its northern end, while building has taken place on the western side; thus the outline of the northern end of the key has shifted westward. Accurate measurements of some of these changes have been kept but will not be given here.

Besides the banks and keys forming the perimeter of the Tortugas, there are also shoals and some small keys in the lagoon. Some of the shoals, White Shoal for example, lie along the direction of flow of the major tidal currents, although with their ends slightly curving toward the west; others are isolated rocks. That some of the latter may be coral masses is suggested by an experience on Garden Key, where all large rocks examined proved to be dead coral heads.

The rocks composing both Marquesas and Tortugas are known only from superficial examination. The foundation of the Marquesas may be oolite, similar to that found on Boca Grande Key, as some of the bottom samples from them were found to contain hard oolite grains. The keys, however, are composed of calcareous detritus, of which *Halimeda* is an important constituent. There is no important coral growth around the Marquesas. The keys of the Tortugas are mostly composed of the comminuted remains of a considerable variety of organisms that secrete calcium carbonate, mollusca, corals, nullipores, echinoids, etc. The corals here, in contradistinction to the Marquesas, are important. Coral reefs, patches, or heads occur along the west side of Loggerhead bank throughout its entire length and around both its southern and northern ends. Bush Key Reef, southwest of Southeast Passage, is luxuriant, and outer reef patches occur northward of Southeast Passage. Reef corals also occur at many places along the sides of channels within the lagoon. Other species of corals, not so large and massive as those on the reefs proper, live on the shallow flats, where not overwhelmed with detritus. Although it has not been practicable to make for the Tortugas a quantitative estimate of the proportions of material contributed by the different kinds of organisms, corals are rather surely the most important. The Marquesas, therefore, are composed of calcareous detritus mostly of other than coral origin; while the Tortugas are composed of calcareous material, of complex origin but largely due to the activity of corals.

The foregoing remarks may be summarized as follows: (1) Submarine solution has not been instrumental in the formation of the Marquesas or the Tortugas lagoon; (2) the atoll rings are constructional phenomena and were shaped by prevailing currents, those caused by winds, the Florida counter current, and tidal currents. The detrital material on which these agencies have worked in the Marquesas is mostly not of coral origin; while in the Tortugas, although of complex composition,[1] it is largely composed of coral débris.

The relative ages of the Marquesas and Tortugas atolls and their relations to oscillation of water-level will now be briefly considered, and in this connection further attention will be given to the probable relations of corals to the formation of the latter atoll. The Marquesas rim is composed of unconsolidated detrital material and evidently requires no oscillation of water-level for its formation. No indication of change of level during or since the building of the rim around the lagoon was observed.

Such a structure as the Tortugas would scarcely be built in the Florida region through material derived from reef corals without change in depth, as the depth of water in the lagoon, 13 fathoms, and outside the atoll rim, 13 fathoms or more, is greater than that in which the Florida reef species are known to flourish.[2] Other evidence, to be adduced in giving an account of the coastal oscillation, renders it reasonably certain that the Tortugas were initially outlined during subsidence.[3] Certain facts indicate that the history of the Tortugas is more complicated than that of the Marquesas.

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[1] The following literature is cited in this connection:

Vaughan Cornish. On the formation of sand dunes. Geog. Jour. vol. 9, pp. 278–309. 1897.

C. Hedley and T. Griffith-Taylor. Corals of the Great Barrier, Queensland, a study of their structure, life-distribution, and relation to mainland physiography. Austral. Assoc. Adv. Sci. Adelaide meeting, 17 pp., 3 pls. 1907.

F. Wood-Jones. Coral and atolls. London. Lovell Reeve & Co. Ltd. (especially chapters XII and XIII, pp. 253–277). 1910.

James Bryce. South America observations and impressions. New York, Macmillan Co. 1912. [Pp. 58, 59 give a good description of crescentic sand-dunes, "the convex of the crescent always facing the wind," on the great inner plateau of Peru.]

[2] In the following discussion of oscillation the terms "uplift," "depression," and "subsidence" are used with reference to land, but it is recognized that oscillation may be due to negative or positive movement of sea-level independent of crustal movement.

[3] The wells bored on Key Vaca by the Florida East Coast Railway, under the supervision of Samuel Sanford, showed the thickness of the elevated coral reef rock to be 105 feet (= 17½ fathoms), indicating that the Pleistocene barrier reef was formed under conditions of subsidence, similar to those postulated for the initial building of the Tortugas rim.

Most or all of the material above sea-level in the Tortugas is unconsolidated, except an outer crust of some of the beach rock, as observed on Loggerhead Key. Extending beneath the sea-level, however, to a depth of 8 feet or more, is beach rock that appears more indurated. Harder rock was also observed at a depth of 3 or 4 feet under water on Southwest and Northeast keys, the subaerial portions of both have been cut away by wave action, and in places submarine cliffs of hard rock, which has been undercut and overhangs the sides of the channels, are evident at depths of over 20 feet. The presence of an older formation for the living reef corals is clear. It seems probable that this rock was subaerially indurated and then depressed. The probability of this subsidence will be further discussed in a succeeding paragraph, wherein the later movements of the Florida coast line will be more fully considered. It appears that the Tortugas atoll with its inclosed lagoon was outlined previous to the present development of the coral reefs of that area, that it was elevated about 50 feet, and that there has been a subsequent (the latest) depression; the land after the oscillation perhaps lacked 10 or 15 feet of being depressed to its former level, thus leaving the keys standing somewhat higher than before.

It has already been stated that the skeletal remains of corals are not only an important constituent of the Tortugas rocks, but are rather surely the most important contributor to its composition. Whether or not the various keys, banks, and isolated rocks were outlined by coral reefs may not be ascertained, but as coral reefs obey the laws of current-moved detritus, in many instances being the principal single source of the detritus, a positive answer to this question is not essential for the validity of the explanation here presented for the building of the Tortugas. Hedley and Taylor say in their paper on the Great Barrier, referred to in the footnote on page 62:

> The growth of an individual reef is shown to proceed in a regular cycle. If the reef reaches the surface with its axis along the wind, then its shape endures; but if across the wind, then its extremities are produced backward, forming first a crescent, later a horseshoe, and lastly an oval, thus inclosing a lagoon.

SKETCH OF THE GEOLOGIC HISTORY OF THE FLORIDA REEF TRACT.

The probability of elevation and subsidence in the Tortugas area leads to a consideration of evidence from which the later movements of the Florida coast line have been inferred and of conditions antecedent to the development of the living Florida barrier reef. That the last important movement of the Florida east coast was downward from Jacksonville to San Augustine is attested by the submerged channel of the mouth of St. Johns River and the submarine fresh-water springs off San Augustine. Late depression of the west coast is positively shown not only by the forms of Tampa Bay and Charlotte Harbor, but also by submerged channels off shore in that region. As the channels off this coast, however, appear not to affect the 10-fathom curve the depression was probably not so great as 60 feet. The very ragged character of the coast line from Cape Romano to the head of Florida Bay, with its salients, reentrants, and almost numberless small islands lying near shore, as Ten Thousand Islands, separated by an interlocking maze of channels, suggests that the southern end of the peninsula to its very tip has undergone subsidence at no distant date. On keys west of Bahia Honda Sanford noted holes, which, at a distance of a quarter of a mile or more from shore, connect with underground passages containing salt water; and on more easterly keys formed of elevated coral reef rock he observed free openings or cavities that extend to a depth as great as 30 feet below sea-level. The evidence is clear that the keys participated in the uplift and subsequent depression that affected the mainland and that at one time they stood more than 30 feet higher with reference to sea-level than they now do. This uplift and the subsequent depression, according to all available evidence, extended to the Tortugas.

The highest points to which the elevated reef extends are on Windleys Island and Plantation Key, where elevations of 18 feet are attained. Supposing these points to have been at sea-level, an uplift with reference to sea-level, of somewhat more than 48 feet, must have taken place since the elevated reef was formed.

The evidence presented shows that the platform, on the outer edge of which the present barrier reef of Florida is principally growing, has geologically, just antecedent to its present relations to sea-level, stood 30 feet or more higher. It is on this shelf, the last oscillatory movement of which was one of depression, that the living reefs of the Florida barrier have established themselves and have grown. However, although the last earth movement has been downward, the net result of the last oscillation, *i. e.*, uplift followed by subsidence, has been to bring the surface of the submarine shelf nearer to the surface of the sea. Although the building of the platform seaward of the Pleistocene barrier reef needs further study, it is known that the base of the Pleistocene reef is about 100 feet below sea-level. As the reef would not have begun to grow in a marked depression, it is probable that the water seaward of it was not shallower than that at its base. The maximum depth of Hawk Channel is about 7 fathoms, while in places the living barrier reaches the surface of the sea. Therefore, since the initiation of the Pleistocene reef the bottom seaward of it has been built up to a thickness ranging from 70 to 100 feet. As the question, how much of this material may be Pleistocene, can not now be answered, it is not known whether or not the living reef has Pleistocene corals beneath it. However these questions may ultimately be answered, it is clear that both addition of material (upbuilding) and uplift have contributed to raising the surface of the basement of the Recent reef, notwithstanding that the last oscillatory movement carried the land surface downward. The hydrographic charts (see particularly U. S. Coast and Geodetic Chart No. 165, Hillsboro Inlet and Fowey Rocks) clearly show the northward extension of the reef platform beyond the limits of the living reefs, which are merely superimposed on a platform whose existence antedates their own.

The conditions under which the Pleistocene barrier reef was formed may be reconstructed by using the results obtained from the recent geologic investigations on the Florida mainland and the records of the wells bored by the Florida East Coast Railway on Key Vaca. Pliocene deposition was followed by uplift, the ensuing Pleistocene deposits being laid down on the eroded surface of those preceding. The Pleistocene reef, which is 105 feet thick, was therefore formed on a subsiding sea-bottom succeeding the uplift at the end of Pliocene time.

The oscillations of southern Florida with reference to coral reefs may be tabularly expressed as follows:

Oscillations of Southern Florida.

Recent.........	Depression (modern) reefs.
Pleistocene......	Uplift.
	Depression (Pleistocene reefs, parts of which now stand as much as 18 feet above sea-level).
	Uplift.
Pliocene........	Depression (some reef corals but no well-developed reefs).

The results of the recent investigations by Sanford, Drew, Dole, and myself have revealed the salient facts in the geologic history of the tract of the Florida keys and reefs, except the immediate foundation rock of the living barrier reef. Great progress was made by clearly showing the process by which the oolites were formed and by proving that the atoll rims of the Marquesas and Tortugas are not due to the solution of an interior mass of limestone but to constructional geologic processes. The hypothesis that Biscayne Bay was formed by marine solution is also definitely disproven.

This history may be summarized as follows: Pliocene deposition was followed by uplift, which was succeeded by depression; during this Pleistocene subsidence along a curve from the eastern side of Biscayne Bay, first trending southward and then bending westward, a barrier coral reef flourished, separated by a channel from the main bank on which the Miami oolite was forming or had formed in strongly agitated waters. West of the coral reef, on an extensive flat in shoal water, the Key West oolite was formed, while still farther to the westward the Tortugas were outlined under the influence of winds and currents. This period of events was succeeded by the elevation of the entire key region to more than 50 feet above its previous level. This uplift was succeeded by one of depression, lowering the surface 30 feet or more, establishing practically the same relation of the sea-level to land that now prevails. Subsequent to the beginning of this last depression the present barrier reef has developed seaward of the keys on a platform already prepared for it, the Marquesas have been formed by winds and currents, and coral reefs have reestablished themselves in the Tortugas.

COMPARISONS OF THE FLORIDA REEF TRACT WITH SOME OTHER CORAL REEF AREAS.

In order to compare the relations of the barrier reef of Florida with the last change of sea-level in Cuba and in Andros Island, Bahamas, the following statements will be made: Hayes, Vaughan, and Spencer[1] have shown, as is evidenced by the pouch-shaped harbors of the Cuban coast and filled channels, such as the submerged filled channel in Havana Harbor, that the last movement of the Cuban coast has been downward with reference to sea-level. As an account of the Cuban reefs would be out of place here, reference is made to that published by A. Agassiz.[2] The platform on which the Cuban reefs grow has been brought to its present position by subsidence. The great barrier reef of Andros Island, Bahamas, as I have shown,[3] occupies the outer edge of a depressed platform. Therefore the Floridian, Andros, and Cuban reefs all have a similar relation to the oscillation of sea-level, as in each instance there has been elevation antecedent to depression which has brought the platforms on which the reefs stand into their present positions.

There is, however, in one important respect, a difference between the relations of the Recent reefs of Florida, Andros Island, Bahamas, and of those of Cuba to Pleistocene oscillation. The oscillations in Florida and Andros Island have taken place without notable differential crustal movements; while in Cuba there was notable deformation accompanying the oscillations. The Pleistocene terraces rise in height toward the eastern end of Cuba in Oriente Province, where altitudes of about 600 feet are attained near Cape Maisí; while in Santa Clara, Matanzas, and Havana provinces terraces on the north side are distinct and rise in several steps to heights of about 200 feet, but are absent or indistinct on the southern side.[4] These facts show that there has been pronounced tilting in late Pleistocene time. In Barbados Pleistocene reefs extend to 1,000 feet in elevation.

By working out the salient features in the development of the Florida reefs and instituting comparisons with the West Indies a basis has been supplied for explaining certain puzzling relations of the reefs in the Tropical Pacific. A. Agassiz discovered that in the Paumotuan atolls the Recent corals were growing as a thin crust on an older limestone foundation. His explanation of the formation of the atolls by the destruction of the interior of a limestone mass must be discarded, as they were certainly not formed in that manner but by constructional agencies. There was evidently a period of atoll formation in the Paumotus previous to the growth of the Recent corals, which have established themselves on an atoll basement already prepared for them. A great development of Pleistocene (and perhaps late Tertiary) coral reefs in the tropical Pacific has been proven in the most convincing way.

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[1] Report on a geological reconnaissance of Cuba, made under the direction of General Leonard Wood, Military Governor, 1901. Report of the Military Governor for 1901.

[2] Bull. Mus. Comp. Zool., vol. 26; pp. 133–136. 1894.

[3] Carn. Inst. Wash. Year Book, No. 11, p. 154, 1912; Carn. Inst. Wash. Pub. 182, page 50, 1913.

[4] Hayes, Vaughan, and Spencer, Geological reconnaissance of Cuba, pp. 18–20.

There is abundant evidence of differential crustal movement in the tropical Pacific similar to that indicated for the West Indies. E. C. Andrews[1] and C. Elschner[2] have both described warping or tilting, the former for the Fijis, the latter for the Pacific more or less in general. Agassiz described an elevated atoll, Makatea, in the Paumotus, and Andrews has given a detailed description of the elevated atoll of Mango, Fijis. The old reefs have been subjected to differentiated earth movement, so that in certain places old atolls, such as Rangiroa and others in the Paumotus, now stand at or near sea-level, while other atolls have been uplifted to heights as great as 230 feet in Makatea and 600 feet in Tuvuthá, Fijis. In other areas there has been depression, Bora-Bora, for instance, as Dana, P. Marshall, and more recently W. M. Davis, have shown.

In conclusion it will be said regarding the Pacific coral reefs: (1) Atolls are not formed by solution, but by constructional geologic agencies; (2) there was in the tropical Pacific a great development of Pleistocene and perhaps late Tertiary reefs which have subsequent to their formation been subjected to extensive differential crustal movement; (3) these deformed older reefs are frequently the basement of the Recent reefs. Although important contributions have already been made to the study of Pleistocene and Recent oscillation and deformation in the Tropical Pacific, there is great need for more extensive and more detailed investigations of this phase of the coral-reef problem in order to ascertain more accurately the relations of the older to the Recent reef series.

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[1] Notes on the limestones and general geology of the Fiji Islands; Bull. Mus. Comp. Zool., vol. 38, pp. 27–30. 1900.

[2] Corallogene Posphat-Inseln Austral-Oceaniens, pp. 15–19, Lübeck. 1913.

V.

SOME CHEMICAL CHARACTERISTICS OF SEA-WATER AT TORTUGAS AND AROUND BISCAYNE BAY, FLORIDA.

BY R. B. DOLE,
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One Map.

SOME CHEMICAL CHARACTERISTICS OF SEA-WATER AT TORTUGAS AND AROUND BISCAYNE BAY, FLORIDA.

BY R. B. DOLE.

INTRODUCTION.

The chemical tests at Tortugas were performed by the writer in June 1913, in the Marine Biological Laboratory, Tortugas, Florida, for the primary purpose of ascertaining what soluble effect, if any, carbon dioxide in sea-water might have on coral and other deposits of calcium carbonate. The tests of waters from Biscayne Bay were made to ascertain the differences in concentration of sea-water in the bay and the diluting effect of Miami River. The samples for the latter work were collected by the writer June 23, 1913, and they were examined during the summer by E. C. Bain, junior chemist, U. S. Geological Survey.

DETERMINATION OF CARBON DIOXIDE.

To 100 c.c. of the sample, in a Nessler tube measuring 17.5 cm. to the graduating mark, 10 drops of 1 per cent phenolphthalein was added and the solution was titrated to an acid reaction by means of N/20 sulphuric acid. This is the usual procedure for estimation of normal carbonates in water. The result of the titration has been calculated to "carbon dioxide (CO_2) present as carbonate (CO_3)" by means of the formula $A = 22Q/d$, in which A represents milligrams per liter of carbon dioxide, Q the number of cubic centimeters of N/20 sulphuric acid required, and d the density of the water.

A sufficient excess of N/20 sulphuric acid was added to 200 c.c. of the sample in a Jena flask and the solution was gently boiled long enough to drive off carbon dioxide, after which the total quantity of acid consumed was determined by titrating the excess with barium hydrate in presence of phenolphthalein. This procedure was followed by Fox[1] and others in determining the alkalinity of sea-water. The "carbon dioxide (CO_2) present as bicarbonate (HCO_3)" was computed by means of the formula $B = 11(Q' - 4Q)/d$, in which B represents milligrams per liter of carbon dioxide, and Q' the total quantity of acid required. The result of the second titration has also been expressed as "total alkalinity in equivalent OH" in order to afford comparison with the results of certain other analysts. This calculation has been made by the formula $C = 4.275Q'/d$, in which C represents the equivalent alkalinity in milligrams per liter of the hydroxyl radicle (OH).

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[1] Fox, C. J. J., On the coefficients of absorption of the atmospheric gases in distilled water and sea-water. Conseil permanent international de la mer, pub. de circonstance 44. February 1909.

DETERMINATION OF CHLORINE.

Chlorine was estimated by means of the salinity outfit supplied by the Copenhagen laboratory of the Conseil permanent international de la mer. This apparatus was obtained through the courtesy of the United States Bureau of Fisheries. The procedure is an adaptation of the usual method of estimating chlorine by titrating with silver nitrate in presence of potassium chromate. An essential feature is a sealed tube of standard sea-water whose content of chlorine has been very carefully determined. This water is used for comparison and the apparatus is so constructed and calibrated as to insure maximum accuracy. Standardized burette No. 8, measuring about 1.5 mm. between graduations, and pipette No. 3, having a capacity of 15.04 c.c., were used. Standard sea-water P_7 2/2, 1912, with a chlorine content of 19.386 grams per kilogram, was titrated frequently during the tests at Tortugas. In the later tests comparison was made indirectly with the same standard by means of a large sample of sea-water from Tortugas that had been titrated several times. Knudsen's correction (k) has been applied to the titrations[1] and salinity (S) has been computed by means of his formula,[1] in which Cl represents grams per kilogram of chlorine: $S = 0.030 + 1.805\ \mathrm{Cl}$.

SPECIFIC GRAVITY.

Density was determined by comparison in 10 c.c. weighing flasks with distilled water at 25° C. Knudsen's values[1] for $\rho_{17.5}$ were used for computing density ($S_{\overline{17.5}}^{17.5}$) as follows $S_{\overline{17.5}}^{17.5} = 1 + \rho_{\overline{1,000}}^{17.5}$, where $\rho_{17.5} = 1.00129\ (0.1245 + \sigma_0 - 0.0595\sigma_0 + 0.000155\sigma_0^2)$ and $\sigma_0 = -0.069 + 1.4708\ \mathrm{Cl} - 0.001570\mathrm{Cl}^2 + 0.0000398\ \mathrm{Cl}^3$. Part of the relatively slight difference between determined and computed figures for sea-water around Biscayne Bay may be attributed to difference of standard; that is, by the determined value the sea-water is compared with distilled water at 25° C., while by the calculated value the specific gravity of the sea-water at 17.5° is referred to distilled water of the same temperature.

SEA-WATERS AT TORTUGAS.

It is believed that solution of calcium carbonate by carbon dioxide in sea-water, if such action takes place, would be shown by regular differences in condition of carbon dioxide in the ebb and flood waters passing out of and into the lagoon surrounded by the shoals and keys of the Tortugas. Accordingly, samples of sea-water were collected twice a day from the middle of Southwest Channel about a mile southeast of Loggerhead Key; these were taken in bottles provided with washered caps that could be clamped down to prevent escape of gases and the samples were kept on ice until they were examined. Those collected from June 11 to 16, inclusive, were tested immediately, and though those taken before the former date had been stored for several days the tests do not indicate that the delay in examination had any appreciable effect on their composition.

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[1] Knudsen, Martin, Hydrographical tables. Copenhagen, 1901.

Table 1.—*Salinity and Carbon-Dioxide Content of Sea-Water from Southwest Channel, Tortugas, Florida.*

Date 1913.	Condition of tide.	Chlorine (Cl).	Salinity (S).	N/20 acid required to neutralize 100 c.c. in the cold (Q).	N/20 acid required to neutralize 200 c.c. after boiling (Q').	Carbon dioxide (CO_2). Present as carbonate (CO_3).	Carbon dioxide (CO_2). Present as bicarbonate (HCO_3).	Carbon dioxide (CO_2). Total.	Total alkalinity expressed in equivalent OH.	Calculated specific gravity $\left(S\frac{17.5}{17.5}\right)$.
		Grams per Kgm.		c.c.	c.c.	Grams per kilogram.				
May 20	Ebbing	19.91	35.97	0.80	9.66	0.018	0.069	0.087	0.0402	1.02748
21	Flowing	19.93	36.00	.92	9.58	.020	.063	.083	.0399	1.02750
	Nearly slack	19.94	36.02	.68	9.54	.015	.073	.088	.0398	1.02752
22	Nearly full	19.94	36.02	.79	9.63	.017	.069	.086	.0402	1.02752
	Ebbing	19.92	35.99	.89	9.88	.020	.068	.088	.0411	1.02749
23	Flowing	19.93	36.00	.96	9.70	.020	.062	.082	.0404	1.02750
	Ebbing	19.91	35.97	.93	9.80	.020	.065	.085	.0408	1.02748
24	Flowing	19.88	35.91	.76	10.07	.017	.075	.092	.0419	1.02743
	Ebbing[1]	19.85	35.86	.51	9.48	.011	.080	.091	.0395	1.02739
25	Flowing	19.89	35.93	.80	10.32	.018	.076	.094	.0430	1.02745
	Ebbing	19.93	36.00	.51	10.26	.011	.088	.099	.0427	1.02750
26	Flowing	......	....	......	......	......	......	......	....	....
	Ebbing	19.92	35.99	.58	9.46	.013	.076	.089	.0394	1.02749
27	Nearly slack	19.93	36.00	.62	9.49	.014	.075	.089	.0396	1.02750
	Ebbing	19.92	35.99	.72	9.50	.016	.071	.087	.0396	1.02749
28	Flowing	19.93	36.00	.70	9.75	.015	.074	.089	.0406	1.02750
	Ebbing	19.93	36.00	.75	9.36	.016	.068	.084	.0390	1.02750
29	Ebbing	19.94	36.02	.75	9.72	.016	.072	.088	.0405	1.02752
	Flowing	19.93	36.00	.83	9.68	.018	.068	.086	.0403	1.02750
30	Ebbing	19.93	36.00	.82	9.28	.018	.064	.082	.0387	1.02750
	Flowing	19.96	36.06	.92	9.53	.020	.062	.082	.0396	1.02754
31	Ebbing	19.94	36.02	.79	9.57	.017	.069	.086	.0399	1.02752
	Flowing	19.94	36.02	.86	9.51	.019	.065	.084	.0396	1.02752
June 1	Ebbing	19.95	36.04	.99	9.41	.021	.059	.080	.0392	1.02753
	Flowing	19.97	36.08	.94	9.44	.020	.060	.080	.0394	1.02756
2	Ebbing	19.95	36.04	.82	9.37	.018	.065	.083	.0391	1.02753
	Flowing	19.94	36.02	.88	9.43	.019	.063	.082	.0393	1.02752
3	Ebbing	19.94	36.02	.80	10.21	.018	.075	.093	.0425	1.02752
	Flowing	19.96	36.06	.70	9.45	.015	.071	.086	.0394	1.02754
4	Ebbing	19.96	36.06	.89	9.34	.020	.062	.082	.0389	1.02754
	Flowing	19.95	36.04	1.04	9.72	.022	.060	.082	.0405	1.02753
5	Ebbing	19.97	36.08	.77	9.54	.017	.069	.086	.0398	1.02756
	Flowing	[2]19.50	[2]35.23	.80	9.93	.018	.072	.090	.0413	1.02691
6	Flowing	19.95	36.04	.75	9.45	.016	.069	.085	.0394	1.02753
	Ebbing	19.95	36.04	.85	9.59	.019	.066	.085	.0400	1.02753
7	Flowing	19.93	36.00	.79	9.45	.017	.067	.084	.0394	1.02750
	Ebbing	19.94	36.02	.77	9.45	.017	.068	.085	.0394	1.02752
8	Flowing	19.95	36.04	.79	9.64	.017	.069	.086	.0402	1.02753
	Ebbing	19.95	36.04	.81	9.55	.018	.067	.085	.0398	1.02753
9	Ebbing	19.95	36.04	.77	9.46	.017	.070	.087	.0394	1.02753
	Flowing	19.96	36.06	.73	9.46	.016	.068	.084	.0394	1.02754
10	Flowing	19.94	36.02	.61	9.55	.013	.076	.089	.0398	1.02752
	Ebbing	19.94	36.02	.54	9.52	.012	.079	.091	.0397	1.02752
11	Flowing	19.98	36.09	.58	9.43	.013	.076	.089	.0393	1.02757
	Ebbing	19.98	36.09	.94	9.54	.020	.062	.082	.0398	1.02757
12	Nearly slack	19.96	36.06	.80	9.86	.018	.071	.089	.0410	1.02754
	Flowing	19.97	36.08	.80	9.44	.018	.067	.085	.0394	1.02756
13	Ebbing	19.92	35.99	.74	9.49	.016	.070	.086	.0396	1.02749
	Flowing	19.92	35.99	.77	9.23	.017	.066	.083	.0385	1.02749
14	Ebbing	19.93	36.00	.88	9.41	.019	.063	.082	.0392	1.02750
	Flowing	19.93	36.00	.77	10.04	.017	.075	.092	.0418	1.02750
15	Ebbing	19.85	35.86	.71	9.33	.016	.069	.085	.0389	1.02739
	Flowing	19.86	35.88	.80	9.56	.018	.068	.086	.0399	1.02741
16	Ebbing	19.91	35.97	.68	9.42	.015	.072	.087	.0392	1.02748
Highest ebbing		19.98	36.09	.....	......	.021	.088	.099	.0427	1.02757
Lowest ebbing		19.85	35.86	......	......	.011	.059	.080	.0387	1.02739
Average ebbing		19.932	36.01	......	......	.017	.070	.087	.0399	1.02750
Average deviation ebbing		±.02	....	......	......	±.002	±.004	±.003	....	....
Highest flowing		19.98	36.09	......	......	.022	.076	.094	.0430	1.02757
Lowest flowing[2]		19.86	35.88	......	......	.013	.060	.080	.0385	1.02691
Average flowing		19.936	36.01	......	......	.018	.068	.086	.0401	1.02749
Average deviation flowing		±.02	....	......	.....	±.002	±.004	±.002	....	....
Highest entire series		19.98	36.09	......	......	.022	.088	.099	.0430	1.02757
Lowest entire series		19.85	35.86	......	......	.011	.059	.080	.0385	1.02691
Average entire series		19.934	36.01	......	......	.017	.069	.086	.0400	1.02750
Average deviation entire series		±.02	....	......	......	±.002	±.004	±.003	±.0008	....
Composite flowing		....	....	.72	9.53	.016	.071	.087	.0399	....
Composite ebbing		....	....	.65	9.60	.014	.075	.089	.0400	....

[1] After heavy shower lasting one hour.

[2] Two determinations; omitted from summary.

SALINITY.

The data in table 1 show that there is no significant or regular difference in chemical composition of the incoming and the outgoing tide. The average chlorine content of the flood water is 19.936 grams per kilogram and the maximum range is only 0.12 gram per kilogram, while the content of the slack water is 19.932 grams per kilogram with a maximum range of 0.13 gram per kilogram. The close agreement of these averages, the narrow range of the values, and the insignificant differences between daily tide contents indicate that there was no essential difference in concentration of the waters during the period in which the samples were collected. These results, with those in table 2, show, however, that the salinity of the sea-water at Tortugas is slightly greater than that of average ocean-water. This greater concentration is doubtless due to high rate of evaporation in the comparatively shallow water.

TABLE 2.—*Chlorine Content and Salinity of Sea-Water at Tortugas, Florida.*

[Grams per kilogram.]

Source.	Date.	Chlorine.	Salinity.
Off Loggerhead Key[1]	1910.	19.60	35.41
Reef, Loggerhead Key[2]	June 1912	19.95	36.04
Southwest Channel[3]	Average for May and June 1913	19.93	36.01
Do.[2]	June 1912	19.99	36.11
Wharf, Fort Jefferson[2]	Do.	19.99	36.11
Off Garden Key[3]	May 25, 1913	19.84	35.84
Do.[3]	June 14, 1913	19.96	36.06
Moat, Fort Jefferson[2]	June 1912	20.09	36.29
Do.[3]	During rain, Jan. 27, 1913.	19.61	35.43
Do.[3]	After rain, Jan. 27, 1913	19.60	35.41
Do.[3]	During rain, Feb. 27, 1913	19.83	35.82
Do.[3]	After rain, Feb. 27, 1913	17.79	32.14
Do.[3]	May 25, 1913	19.67	35.53
Do.[3]	June 14, 1913	19.83	35.82
Do.[3]	June 15, 1913	19.19	34.67
Content of ocean water[4]		19.386	35.02

[1] Computed from report of analysis by G. Steiger, of the U. S. Geological Survey Laboratory; published in The Data of Geochemistry, by F. W. Clarke, Bull. U. S. Geological Survey, 491, p. 113, 1911.
[2] Tested at the Plymouth (England) laboratory.
[3] Tested by R. B. Dole.
[4] Standard P_7 2/2, 1912.

ALKALINITY.

The average alkalinity of water from the Southwest Channel, 0.0400 OH gram per kilogram, is that given by Allen and others for normal sea-water.[1] The average alkalinity of the flood water, 0.0401, and that of the slack water, 0.0399, differ from each other much less than the average deviation of individual observations (±0.0008), and agree with the estimates of alkalinity of the composite flood- and slack-tide samples recorded in the last two lines of table 1. The alkalinity of these waters seems to be practically the same as that of normal sea-water.

The average total content of carbon dioxide of the incoming water is essentially that of the outgoing water, and though the recorded values differ somewhat from day to day the range is not very great or very significant; there is no regular excess of the values of one set over the other. Though the probable error of individual determinations, about 0.0020 gram per kilogram, is less than some of the differences, the variations are about what might be expected in a series of examinations of a flowing water.

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[1] Allen, E. J., and E. W. Nelson. On the artificial culture of marine plankton organisms. Quart. Journ. Micros. Science, vol. 55, pt. 2, June 1910.

The quantity of acid required to neutralize the cold sea-water in presence of phenolphthalein proves conclusively that *"free" carbon dioxide, in the ordinary acceptance of that term in America, is not present.* Consequently, the inflowing waters at Tortugas would have no soluble action on deposits of calcium carbonate by virtue of the carbon dioxide they contain. One-half the carbon dioxide in equilibrium as the bicarbonate radicle (HCO_3) is commonly termed the "half-bound" carbon dioxide and that quantity is probably the carbon dioxide referred to as "free" by some writers on the composition of sea-water. So far as can be ascertained from the literature the carbonate radicle (CO_3), but not the free acid, is always present in sea-water; or, as Dittmar expressed it in his computation of hypothetical combinations, the carbon dioxide is more than sufficient to complete the formation of the carbonate but considerably less than is required to form the bicarbonate.[1] Dittmar reports[2] the average alkalinity of 130 samples of ocean water as 54.70 milligrams per liter of CO_2, meaning thereby the combined carbon dioxide (total minus the half-bound acid); the corresponding figure computed from the results at Tortugas is 52.5 milligrams per liter.

Briefly, it may be stated that the condition and the quantity of carbon dioxide in the water entering Tortugas Harbor do not appear to be essentially different from those of the escaping water or normal sea-water. *The inflowing water has no apparent action on deposits of calcium carbonate by virtue of its content of carbon dioxide.*

SEA-WATER AROUND BISCAYNE BAY.

HYDROGRAPHY.

Biscayne Bay ranges from 3 to 8 miles in width and extends north and south of the mouth of Miami River, though lying mostly south of it. It is bounded on the east by low keys and reefs, through which there are numerous narrow channels. It is very shallow, being for the greater part only 8 to 12 feet deep. Featherbed Bank, a shoal, cuts off the northern part of the bay, and coral banks covered with sand extend from Ragged Keys to Key Biscayne. Miami River is a small stream, discharging only about 400 second-feet at the time of sampling.

COMPOSITION OF THE WATER OF MIAMI RIVER.

No analyses of water from Miami River above tidal influence are available, but as it receives drainage from the Everglades its water is doubtless similar in concentration and composition to that of Lake Okeechobee and Fort Lauderdale Canal, analyses of which are quoted in Table 1. These are analyses of calcium-carbonate waters of low mineral content carrying considerable organic matter. Normally Miami River may be expected to contain a small amount of free carbon dioxide and no carbonate (CO_3).

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[1] Quoted by Fox, C. J. J. Conseil permanent international de la mer, pub. de circonstance 44. February 1909.

[2] Dittmar, William. The alkalinity of ocean water. Report of the voyage of *H. M. S. Challenger* 1873–76, vol. 1, p. 124.

TABLE 3.—*Composition of Drainage from the Florida Everglades.*[1]

[Milligrams per liter.]

	North New River Canal, at lock 6 miles above Fort Lauderdale.	Lake Okeechobee, 3 miles north of Ritta Island.
Total solids at 180° C.	130	183
Total solids at dull red heat	126	143
Organic and volatile matter	4	40
Silica (SiO_2)	8.2	8.2
Iron (Fe)	.03	.03
Calcium (Ca)	26	31
Magnesium (Mg)	6.7	7.0
Sodium (Na)	19	16
Potassium (K)	2.0	2.0
Carbonate radicle (CO_3)	.0	4.8
Bicarbonate radicle (HCO_3)	94	104
Sulphate radicle (SO_4)	4.1	7.3
Chlorine (Cl)	26	28
Nitrate radicle (NO_3)	.1	.1
Color	32	41

[1] Samples collected in June 1913, by R. B. Dole; analyzed by W. T. Read.

TESTS OF BAY WATERS.

Samples were collected in or just outside Biscayne Bay at the points shown on the accompanying map and the chemical tests of them are recorded in table 4.

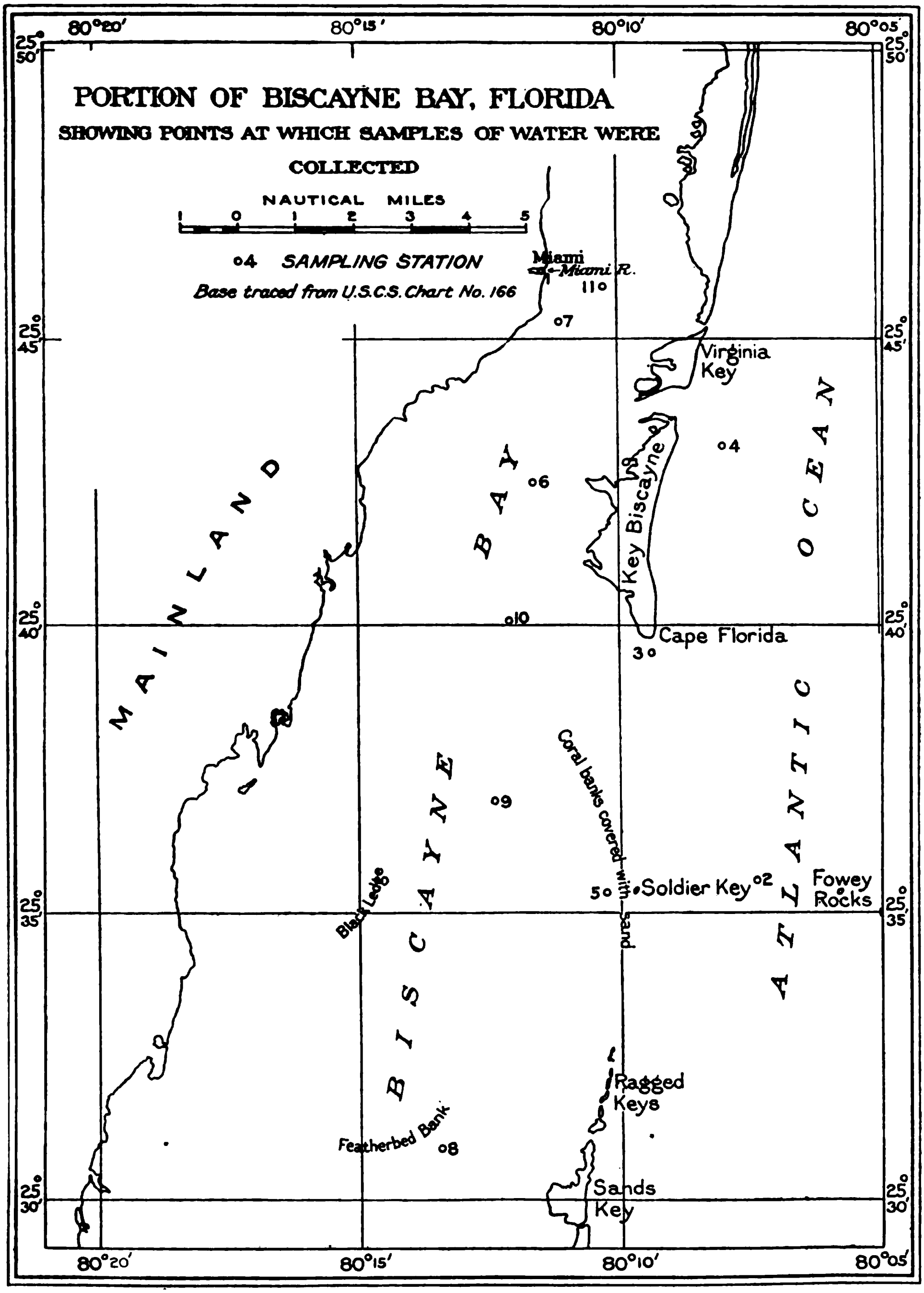
TABLE 4.—*Some Chemical Characteristics of Sea-Water Around Biscayne Bay, Florida.*

[Samples collected June 23, 1913, by R. B. Dole; analyses by E. C. Bain.]

No.	Location.	Temp. of water.	Time.	Carbon dioxide (CO_2) present as carbonate (CO_3).	Carbon dioxide (CO_2) present as bicarbonate (HCO_3).	Total alkalinity in equivalent OH.	Chlorine.	Calculated salinity.	Specific gravity calculated (17.5°).	Specific gravity determined (25°).
		°C.		Grams per kilogram.						
1	Mouth Miami River inside bar near Royal Palm dock	26.3	4^h30^m a.m.	Tr.	0.232	0.078	1.42	2.59	1.0020	1.0023
11	1.5 miles east of Miami at 11 ft. depth in government channel north of Virginia Key	27.3	2 00 p.m.	0.022	.068	.043	14.73	26.62	1.0203	1.0197
7	Off Cormorant Point (tide ebbing)	26.6	4 45 a.m.	.018	.091	.049	13.35	24.13	1.0184	1.0184
6	1 mile west of west point of Key Biscayne near inside P & O marker	27.2	5 30	.013	.083	.043	16.80	30.35	1.0232	1.0228
10	0.25 mile west of Old Man Beacon	26.0	6 00	.023	.064	.043	18.79	33.95	1.0259	1.0256
9	3 miles northeast of Black Ledge	27.3	7 00	.026	.047	.038	20.33	36.73	1.0281	....
5	0.5 mile west of Soldier Key	27.0	9 00	.018	.064	.039	20.28	36.64	1.0280	1.0279
8	In channel through Featherbed Bank	26.4	8 00	.018	.037	.028	20.28	36.64	1.0280	1.0278
2	1.5 miles west of Fowey Rocks Light, outside bay	26.9	11 00	.026	.083	.053	19.93	36.00	1.0275	1.0270
3	In channel south of Key Biscayne and 0.5 mile south of Old Florida Cape Light, outside bay	27.2	11 45	.026	.033	.032	19.83	35.82	1.0274	....
4	At red buoy outside bar across entrance to Bear Cut, a mile southeast of Virginia Key; outside bay	27.7	1 15 p.m.	.026	.055	.042	19.99	36.11	1.0276	1.0274

DETERMINED AND CALCULATED DENSITIES.

No particular importance can be given to the relatively slight differences between the determined and calculated densities, especially in view of the absence of several duplicate determinations on each sample. On the other hand, the fairly close agreement of the estimates indicates that the density of sea-water, even when largely diluted by river water, may be used in


PORTION OF BISCAYNE BAY, FLORIDA
SHOWING POINTS AT WHICH SAMPLES OF WATER WERE COLLECTED
NAUTICAL MILES
1 0 1 2 3 4 5
o4 SAMPLING STATION
Base traced from U.S.C.S. Chart No. 166
80°20′
80°15′
80°10′
80°05′
25° 50′
25° 45′
25° 40′
25° 35′
25° 30′
Miami
Miami R.
11o
o7
Virginia Key
o4
Key Biscayne
o6
o10
Cape Florida
3o
MAINLAND
BAY
BISCAYNE
OCEAN
ATLANTIC
Coral banks covered with sand
o9
5o
Soldier Key
o2
Fowey Rocks
Black Ledge
Ragged Keys
Featherbed Bank
o8
Sands Key

place of the directly determined density in computation of the chemical analysis. Obviously, however, the relation could not be depended upon for inland saline solutions, which vary greatly in the percentage composition of their anhydrous residues.

DIFFERENCE IN CONCENTRATION.

The salinities of the three samples taken outside the reefs (2, 3, and 4) agree closely with each other and with the salinity of gulf water at Tortugas, Florida (36.01), which is somewhat greater than that of standard ocean water (35.02). The water in the south part of the bay is somewhat more concentrated, samples Nos. 9, 5, and 8 having salinities of 36.73, 36.64, and 36.64, respectively. This evidence that the water in this part of the bay is concentrated by evaporation during its retention in the shallows serves further to indicate that circulation there is not very rapid and that the greater bulk of the water inside the keys is not thoroughly mixed or shifted by the tides. The inside samples only as far south as Old Man Beacon give evidence of dilution by fresh water; therefore it may be concluded that, at least at the time these samples were collected, the effect of Miami River on the water of the bay did not extend south of Soldier Key nor outside the keys. Sample 1 has a salinity obviously higher than the pure water of Miami River alone may be expected to have, and represents admixture with bay water; carbonates are absent from it, but bicarbonates are much higher than in the normal drainage from the Everglades and may be attributed to reaction of the carbon dioxide that the river water carries. In all other samples normal carbonates are present as in sea-water and *free carbon dioxide is entirely absent*, but there are wide differences in the alkalinity from point to point.

VI.

OBSERVATIONS UPON THE GROWTH-RATE AND OECOLOGY OF GORGONIANS.

BY LEWIS R. CARY,

Instructor in Biology, Princeton University.

Two plates.

OBSERVATIONS UPON THE GROWTH-RATE AND OECOLOGY OF GORGONIANS.

By Lewis R. Cary.

The following account includes the record of observations, extending over a period of three years, upon the growth-rate of three species of gorgonians under the natural environmental conditions found on the reefs about Dry Tortugas, Florida. The œcological data have been secured in the same periods of observation and are supplemented by observations made at Montego Bay, Jamaica.

RATE OF GROWTH OF GORGONIANS.

The records of the growth-rate of *Gorgonia flabellum* and *Plexaura flexuosa* are for specimens occurring on the reefs immediately surrounding Loggerhead Key, the location of the Tortugas laboratory. All of the specimens have been allowed to remain in their original place of attachment except those shown in the accompanying plates, the measurements of which are given in table 2. These were cemented to tiles in order that they could be more easily photographed from time to time to afford a visual as well as a linear record of their growth.

Table 1.

Specimen No.	Gorgonia flabellum.					
	Height.				Increase from—	
	June 1910.	Jan. 1911.	June 1911.	Aug. 1912.	1910–11.	1911–12.
	mm.	*mm.*	*mm.*	*mm.*	*p. ct.*	*p. ct.*
1	[1]100	135	150	gone	50	....
2	[1]100	138	150	155	50	3.33
3	[1]70	100	145	183	107.14	26.20
4	300	....	300	374	10	10.30
5	150	....	219	245	46.38	12.38

Specimen No.	Plexaura flexuosa.					
	Height.				Increase from—	
	June 1910.	Jan. 1911.	July 1911.	Aug. 1912.	1910–11.	1911–12.
	mm.	*mm.*	*mm.*	*mm.*	*p. ct.*	*p. ct.*
1	80	105	135	175	68.77	29.62
2	150	....	155	210	3.3	35.48
3	50	65	83	101	66.66	21.68
4	245	....	269	287	9.7	6.69
5	115	150	163	183	41.39	12.27

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[1] These specimens had been exposed to the air for a long time during an exceptionally low tide on June 6, 1910, so that the height given in the table does not represent the actual growth since attachment, but the amount living at that time.

The most complete records of the growth of specimens of *G. flabellum* and *P. flexuosa* among my data are those for some specimens growing upon a single coral head (*Orbicella annularis*) some 12 feet in diameter, situated about 200 yards west from the laboratory upon a shallow reef. Table 1 shows the growth-record for the period since June 1910, and gives the original size and the change in height of the same specimens of gorgonians after 7 months, 1 year, and 2 years from the time of first measurement; with the percentage of increase in height for the 2 years.

Similar records of individuals of the same species growing upon tiles, photographs of which constitute a part of this report, are shown in table 2.

TABLE 2.—*Heights of gorgonians attached to tiles to show growth in a year.*

Tile and species.		July 1911.	Aug. 1912.	Per cent increase.
		mm.	*mm.*	
Tile 1	G. flabellum	65	148	127.69
	P. flexuosa	130	150	15.38
Tile 2	P. flexuosa (*a*)	145	175	20.62
	P. flexuosa (*b*)	70	120	70.43
Tile 3.	G. flabellum	41	124	202.43

All of the specimens recorded in table 2 were selected without any special reference to size, as at that time no data were at hand to afford the basis for a correlation between size and age. Small (young) individuals of either of the above-listed species were so rarely found on the reefs during the period of observation in June 1910 or July 1912 that, although the specimens on any of the reefs studied could be readily arranged into a series of groups, each containing individuals of about the same size, the relation in respect to age among the several groups could not be determined with certainty.

The study of the same areas of reef in August 1912 showed the presence of a considerable number of small specimens of both species. On the coral head west of the laboratory there was found on August 21, 1912, seven specimens of *P. flexuosa*, which had become attached, or at least had become noticeable, since July 23, 1911. The length of these specimens, in order of magnitude, was 25 mm., 26 mm., 31 mm., 32 mm., 42 mm., 42 mm., and 46 mm., respectively. Beside these there was found a single young specimen 3 mm. in length, which consisted of only two calyces and polyps. The identification of this specimen as *P. flexuosa*, which is based upon the character of the spicules and that of the orifice of the calyx, is at best uncertain. The specific identity of the specimen is of considerable interest in connection with other data bearing on the time and duration of the breeding season, as will be pointed out in the section of this paper dealing with œcology.

On a shallow reef east of Loggerhead Key, where measurements of all gorgonians had been made yearly since 1910, there were found 15 specimens of *P. flexuosa* less than 50 mm. in length. These were not of a recognizable size, if present, when the measurements were made over the same area in July 1911.

Upon the last-mentioned reef 26 specimens of *G. flabellum*, less than 65 mm. in height, were also found, which were not recognizable when the measurements were made in July 1911.

On the basis of these observations, in conjunction with the records given in tables 1 and 2, it becomes possible to make the records complete for at least two years in the case of *G. flabellum* (fig. 8, plate 2). Observations after another year's growth will, moreover, fill in the stages to complete the record for six years.

All specimens of *G. flabellum* and of *P. flexuosa*, on a reef east of Loggerhead Key, were measured in July 1911 and again in August 1912. Most of the individuals, as already mentioned, fall into natural "size groups," which for convenience have been designated as follows: Group 1, 0 to 75 mm.; group 2, 75 to 150 mm.; group 3, 150 to 300 mm.; group 4, 300 to 450 mm.; group 5, 450 to 600 mm.; group 6, 600 + mm. As shown in table 3, the percentage of individuals in groups 1 and 2 varied markedly in the two seasons, while the number of the larger specimens was proportionately similar for the two years.

TABLE 3.—*Size groupings as shown by measurements of all individuals on a single shallow reef.*

Species and date.	Group 1.	Group 2.	Group 3.	Group 4.	Group 5.	Group 6.
	p. ct.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
G. flabellum, 1911	0	7.35	36.76	30.88	16.17	8.82
G. flabellum, 1912	15.4	10.61	29.70	22.02	18.45	3.59
P. flexuosa, 1911	0.62	8.06	45.16	37.09	6.54	0.62
P. flexuosa, 1912	8.33	17.77	44.44	23.88	5.00	0.55

The observations upon the breeding season and the attachment of the planulæ are intimately connected with those in relation to the absence of small (young) specimens of gorgonians during the seasons of 1910 and 1911. In June 1910 almost every colony of *P. flexuosa* examined was carrying mature eggs which could be stimulated to development by the use of chemicals which are ordinarily efficacious in bringing about artificial parthenogenesis. None of the planulæ among those to be obtained in the tow net could with certainty be identified as those of this species, nor could any of the colonies be kept long enough in aquaria to secure planulæ. The evidence from artificial parthenogenesis is, however, enough to establish the fact that the breeding season was in progress at that time.

In July 1911 some of the colonies of *P. flexuosa* contained ripe eggs, but the greater number were without apparent gonads. Late in August 1912 not a single specimen was found in which gonads were recognizable, although more individuals were examined to determine this point than in either of the other years. The breeding-season for *P. flexuosa* is, then, apparently at its height in June. The number of individuals with ripe sexual products diminishes rapidly through July, while by the latter part of August the breeding season is over for any given year. On August 21, 1912, a young specimen, probably of *P. flexuosa*, was found, the size of which (3 mm. in height) was such that it would have been overlooked had it not been growing in a location where its color was in striking contrast to the background of white coral.

To apply these facts to the observed scarcity of yearling specimens in 1911, which was a general occurrence over all of the reefs visited in the regions about Tortugas, it is necessary to take into account a severe hurricane which occurred on October 16, 1910. The destruction of adult gorgonians by this storm is known to have been very severe upon certain reefs and the amount of débris moved over the bottom in shallow water was very great, which in itself would account for the destruction of the very young specimens at that time. In July 1911 there would be no easily recognizable young which had set during the same season, and only very rarely had any of those from the set of the year before been able to survive the destructive effects of the hurricane. In August 1912, on the other hand, young specimens of several gorgonians, besides the two above discussed, were found upon every considerable reef examined. This would seem to show that the setting of a considerable number of young was the usual occurrence. That the number of young found during the past season on the reefs, which had been carefully examined each season for three years, may be greater than would ordinarily be found might be inferred from the breeding habits of some other sedentary invertebrates, especially the Mollusca, where after a season, when for any cause the set is below normal, an especially heavy set is to be expected the following year.

ATTACHMENT OF PLANULÆ.

Vaughan's observations on the attachment of coral planulæ[1] show that for effective attachment they must settle in a place protected from too rapid currents and wave-action, where the bottom is rough, and where other more rapidly growing organisms are least likely to obtain a foothold.

For the effective attachment of the planulæ of the gorgonians studied the roughness of the bottom, *i. e.*, the presence of small depressions or cracks into which the planulæ could settle, appears to be the most important factor. In every instance the one-year old specimen had its base in a depression. Frequently the depth of the point of attachment below the surface of the rock was more than half the length of the colony. The single (very small) specimen found, when it consisted of only one or two calyces, was entirely below the level of the surface of the coral head to which it was attached. Many of the adult colonies have their point of attachment at the bottom of a crack of considerable depth, so that, on the basis of the observed growth for a year, they must have remained below the general level of the bottom until more than one year old. In the later growth of the gorgonian colony its non-polyp-bearing basal portion spreads beyond the limits of the crack over the bottom, so that its apparent height is not measured from its original point of attachment.

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[1] Carnegie Institution of Washington Year Book No. 10, pp. 151–152.

On the shallow reefs, where the gorgonians are most abundant, the amount of algæ, bryozoons, and other incrusting organisms appears to vary only slightly during the different seasons of the year. In no observed instance did these organisms seem to form a mass dense enough to exclude a sufficient amount of water for the well-being of the young gorgonian colony. Frequently the fronds of algæ extend beyond the free ends of the gorgonian colony, but the specimens found in such locations appear, to judge from comparative measurements, to have suffered no harm from being so closely surrounded.

In comparison with the young coral polyp the gorgonian colony has an obvious advantage in that its most rapid growth is perpendicular to the surface of the substratum, which would keep the most rapidly growing part in a position favorable for the securing of food and oxygen.

On the sea-bottom, away from the shallow reefs, gorgonians are generally found as scattered specimens or in groups of at most only a few individuals. These colonies will almost invariably be found to be attached to some irregular mass of rock which affords the protecting cavity necessary for the permanent attachment of the young gorgonian.

DESTRUCTIVE AGENTS.

Of the destructive agents to which the gorgonians are subjected the wave-action during severe storms is apparently by far the most important. The wave-action developed by the ordinary northeast trade-winds during the winter months is of sufficient force to cause the destruction of many colonies which have a comparatively weak attachment. The tendency for any colony to be torn away will increase from year to year as its surface area becomes increasingly larger.

An opportunity to study wave-action at its maximum severity was afforded the writer in January 1911, and later, in July of the same year, when the observations made during the first visit were confirmed and extended to a much greater area. The hasty observation made in January shows that, as the result of the hurricane of the previous October, there had been a great destruction of gorgonians on the reefs about Tortugas. On the east shore of Loggerhead Key, the only one visited at that time, many specimens of some five or six species of gorgonians were found thrown up on the beach along its entire length. At that time no estimate of the number cast up was made nor was the area visited sufficient to give conclusive evidence of the proportion of colonies carried from their natural location. In July 1911 the observations on these points were extended to cover a large area of shore-line and submerged reef.

At one point on the inside of the east reef, near Bush Key, the gorgonian skeletons were counted over a strip of beach 112 yards in length, where there was a windrow, perhaps a yard wide, made up of these skeletons. The number of skeletons in a linear yard (it was practically a square yard) averages 75.7 for ten counts made at approximately equal distances through the above-mentioned distance, or nearly 8,500 colonies for the whole area. This area showed very clearly that the most destructive part of the storm came from nearly northeast. On the outside of the reef, in the direction indicated by the wash of the storm, only two living colonies of gorgonians were found for as far off-shore as the water was sufficiently shallow to allow one to wade about over the reef. Gorgonians were growing abundantly over this area when it was visited in 1910, so the destruction had been almost complete. That the great number of the colonies found in the windrow on the beach had come from this shallow-water area was shown by the fact that in deeper water on the outer portion of the reef there was little evidence of the lessening of the number of colonies below the normal number for such locations; the colonies here are always comparatively scattered, never forming dense "thickets," as they do on the reefs in shallow water. The very shallow water on the outside of the reefs contained a considerable number of skeletons of *Gorgonia flabellum* and *Plexaura flexuosa*, which had been broken off from their supports and carried away from their original location while still attached to a good-sized piece of coral rock. Apparently these specimens had reached their present location at a time when the wave-action had become insufficient to carry them over the crest of the reef onto the beach on the lagoon side. By far the larger number of specimens on the beach, on the lagoon side of the reef, were still attached to a piece of coral rock, usually of small size. In almost every instance the skeleton shows that the colony was complete when washed on shore. Any physical injury undergone had not been to the extent of having branches broken off or, in the case of *G. flabellum*, having suffered any tearing of the blade-like portion of the colony.

On the east side of Loggerhead Key, where the greatest force of the storm came across comparatively shallow water, the reef just mentioned being about 3 miles distant, most of the specimens, when examined in January 1911, had the usual spicule-bearing tissues present, although considerably macerated in many instances. None of these colonies showed any considerable amount of injury, such as the loss of branches or the tearing of the living tissue from the skeleton.

None of the common gorgonians of the Tortugas region can be kept alive for any considerable time after they have been broken off from their natural support and allowed to fall over into a horizontal position. When such a colony is put into a live-car, where most of the other marine invertebrates and practically all of the sedentary Cœlenterates can be kept alive for an indefinite period, it will be only two or three days before maceration sets in. It seems apparent, therefore, that the greatest destruction by storms comes from the tearing of the gorgonian colonies from their natural supports rather than from any laceration of the tissues.

On the reef where the measurements previously mentioned were made, a considerable number of colonies were carried away during the storm. Of the remaining colonies, many were found in January 1911 which had suffered laceration to a considerable extent. The most common injury observed was a destruction of the living tissues of the colony, such as would result from twisting a specimen in one's hand while holding it firmly by each end. The loss of branches in the branched forms, or the tearing of the skeleton of the leaf-like portion of *G. flabellum*, was a very unusual occurrence. The injuries caused by the twisting from the wave-action were quite evenly distributed among the different species growing on this reef. Such injuries often involve as much as half the total surface-area of each colony. In all large colonies, of whatever species, the injury was greatest over the basal portion, while the outer end or branches were usually uninjured. At the time of the examination in January 1911 there had been comparatively little ingrowth of new tissue over the denuded areas, so that the extent of the injury to any colony could be readily determined.

When the same specimens were re-examined in July 1911, in at least 50 per cent of the specimens noted as injured at the time of the earlier observations, the reparation had been so complete that there was no longer any evidence of injury. In all of the colonies where the injury consisted in the removal of living tissue from about the base of the skeleton, there was yet an area where the skeleton was exposed. In all cases where the injury was of this nature there was no evidence of any growth of the living tissue down over the naked skeleton. The exposed skeleton was usually covered with a dense growth of algæ, bryozoa, or hydroids, so that there seemed to be no probability that it would again be covered with the normal tissues.

The examination of the same individuals in August 1912 showed comparatively little change since the previous summer. In some instances the cœnenchyma had grown down over the naked skeleton for some little distance, but usually the basal portions of the skeleton were, just as at the last examination, covered with algæ, incrusting bryozoa, etc.

The leaving exposed of areas of the skeleton near the base of the colony may have a causal relation to the fact that, on all of the shallow reefs, gorgonians are found which are to a greater or less extent covered by an overgrowth of *Millepora alcicornis*. In most cases the *Millepora* surrounds what was really the basal portion only of a gorgonian skeleton. Not at all uncommonly, however, a colony of *Millepora* is found which covers a skeleton of *Gorgonia flabellum*, in which the mesh-work of the skeleton of the "leaf" could be made out in all its details. Frequently the relation between the height and width of the colony, as well as the characters of its outline, showed that the *Gorgonia* had not suffered any disintegration before being covered by the *Millepora*. As in a small number of instances the *Millepora* was observed growing up about the naked skeleton at the base of the *Gorgonia* colony, and as the complete naked skeleton of such a colony is very rarely found in its natural attachment on the reef, it seems almost certain that the *Millepora* must have, on account of its more rapid growth, covered over and destroyed the living tissue of the colony.

A skeleton thus incrusted with *Millepora*, which was complete in structural detail in July 1911, had by August 1912 been reduced to about one-third of its original height. Nothing of the *Gorgonia* skeleton beyond the stouter branches remained. The whole of the "leaf" had been broken down, presumably by the weight of the incrusting *Millepora*.

The previously mentioned hurricane was, as had been pointed out in dealing with the growth-records, sufficiently prolonged to destroy almost every young gorgonian which had become attached during the breeding-season of that year. No evidence could be secured to determine whether the destruction took place by an actual washing of the colonies from their attachment or by the shifting of the easily movable material—algæ, sediment, shells, and even quite large fragments of coral rock—which by filling up all cracks in the surface of the reef would have been sufficient to smother the small individuals.

Gorgonians growing upon the shallow reefs are, with more or less regularity, subjected to exposure to the air. In summer the uncommonly low spring-tides (when the low full-moon tide comes in the late afternoon) most frequently come on calm days, when the exposure is most destructive. On June 6 and 8, 1910, and again on July 23, 1911, all of the shallow reefs were exposed for a space of time sufficient to destroy the superficial tissues of most corals and gorgonians which grew on the highest part of the reefs. While all of the species of gorgonians studied suffered to a greater or less extent, the injuries were most severe in the case of *G. flabellum* and *G. acerosa*. Frequently there resulted the death of the distal half of the colony which, within a few days after its exposure to the air, would slough off from the uninjured proximal part. *Plexaura* and *Eunecia* were much less frequently injured, the latter in only two observed instances, and then only after prolonged exposure. The ability of the two last-mentioned forms to withstand the exposure is at least correlated with, if not dependent upon, the thickness and toughness of the cœnenchyma into which the polyps may be retracted when the colony is exposed.

In all observed instances of injury from exposure to the air the uninjured portions of the colony showed no ill effects from the injury of the distal portion and their growth was at least as rapid as in an uninjured specimen.

REGENERATION EXPERIMENTS.

The injuries occurring in nature are so variable and are besides so entirely uncontrollable that a series of experiments was undertaken in order to secure more definite data concerning the capacity for regeneration in these animals. All of the experiments here described were extensive in nature, usually involving skeleton and cœnenchyma as well as the polyps. In short, they were made to simulate the injuries to which the gorgonians are subjected under natural conditions on the reefs.

Table 4 shows the nature and extent of the operations performed upon the gorgonians used in the regeneration experiments and the extent of recovery at different times after the operations.

TABLE 4.

Species and specimen Nos.	Operation June 1911.	Recovery.		
		January 1911.	July 1911.	August 1912.
G. flabellum:				
No. 1....	One-half cut from each leaf (longitudinally).	Healed, no regeneration.	No lateral growth....	No change.
No. 2....	Split lengthwise into 5 pieces.	Edges healed........	0.25 inch lateral growth.	0.5 inch lateral growth.
No. 3....	Circular pieces cut from leaf.	Edges healed........	0.25 inch in growth...	Spaces nearly filled with new tissue.
No. 4....	Cut back one-half (6 in. remaining).	Healed over; had grown to 7.5 inches.	9.5 inches long, distal end of leaf rounded.	12.25 inches long.
P. flexuosa:				
No. 1....	Side branches cut off, central ones slabbed on one side, exposing the skeleton.	Had healed over the cut places; new polyps appearing in the scar-tissue.	Polyps now cover all stripped areas. New branches (8) have come in on stumps of cut branches.	No change.
No. 2....	Cut back from 11 inches to 6 inches.	Ends of branches healed over. Now 7.25 in. high.	Now 10 inches high...	13 inches high.
G. acerosa...	One side of all branches trimmed and slabbed.	No new branches. Scar-tissue covers all wounds. No polyps on scar-tissue of larger branches.	Polyps have appeared in scar-tissue on all smaller branches but not on any of the larger branches.	No change.

Pieces of the regenerating branches of *G. acerosa* were preserved when the reefs were visited in January 1911 and sections of this material cut and studied. In this species there is always found a complete layer of cœnenchyma over a denuded area which remains free from polyps for some time. The entodermal canals keep pace with the formation of the other tissues, but it is only after a considerable time that the bud-like swellings of the canal, which mark the points of origin of new polyps, make their appearance. They always follow the same sequence as that shown in the formation of new tissue over a denuded area, appearing first at the periphery of the wound nearest to the uninjured polyps. The formation of new skeletal tissue at the cut ends of the branches takes place very slowly at first. After the new rod of skeletal tissue has reached a diameter equal to that of the older portion the elongation of the branch takes place rapidly. If the living tissue be removed from about the base of the colony, there is, so far as my observations go, little down-growth of the tissues from the cut surface over the naked skeleton.

EXPLANATION OF PLATES.

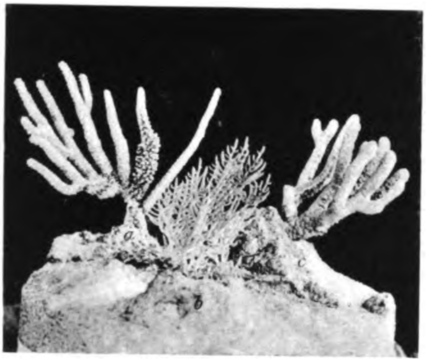
The disks to which the specimens photographed are attached are 8 inches in diameter. While the reduction is not exact in every instance, the diameter of most of the disks in the reproductions is about 2 inches, making the specimens approximately 0.25 natural size.

PLATE 1.

1. Tile 1. A, *Plexaura flexuosa;* B, *Gorgonia flabellum;* C, *Eunecia* sp. Photographed August 1911.
2. Tile 1. Specimens same as above. Photographed August 1912.
3. Tile 2. A and B, two specimens of *Plexaura flexuosa.* Photographed August 1911.
4. Tile 2. Same as in figure 3. Photographed August 1912.
5. Tile 3. A, *Eunecia* sp.; B, *Gorgonia flabellum.* Photographed August 1911.
6. Tile 3. Same. Photographed August 1912.

PLATE 2.

7. Tile 8. A, *Eunecia* sp.; B, *Plexaura flexuosa;* C, *Eunecia* sp. Photographed August 1911.
8. Tile 8. Specimens as in figure 1. Photographed August 1912.
9. Tile 7. A, *Plexaura flexuosa;* B, *Eunecia* sp.; C, *Gorgonia flabellum.* Photographed August 1912. See figure 13.
10. Tile 15. A, *Eunecia* sp.; one year old, B, *Gorgonia flabellum,* one year old. Photographed August 1912.
11. Specimen of *Plexaura flexuosa,* one year old; 0.5 natural size. Photographed August 1912.
12. Tile 16. One-year old specimens of *Plexaura flexuosa.* Photographed August 1912.
13. Tile 7. Specimens as in figure 9. Photographed August 1911.
14. Tile 18. A, One-year old specimen, and B, a two-year old specimen of *Gorgonia flabellum.* Photographed August 1912.



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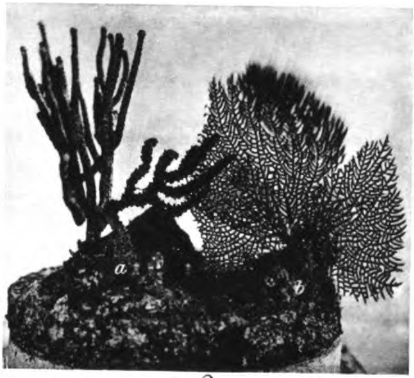

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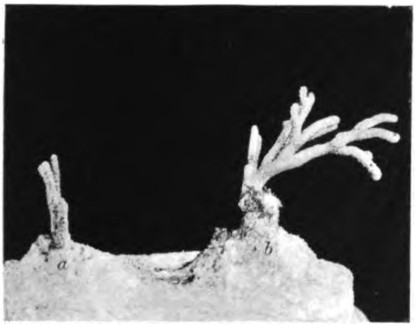

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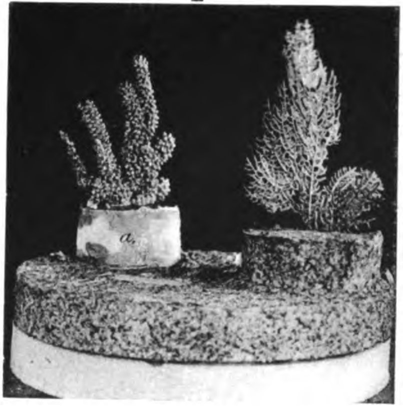

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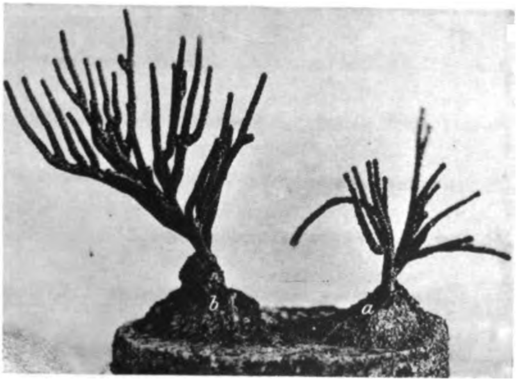

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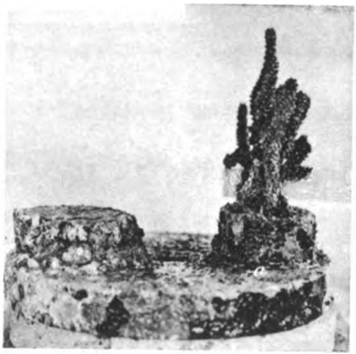

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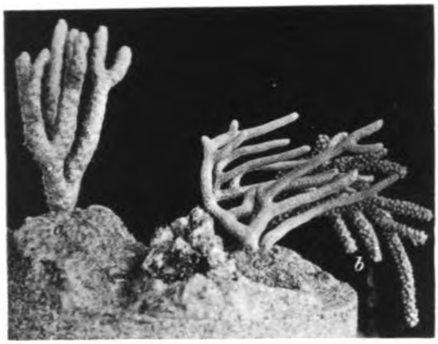

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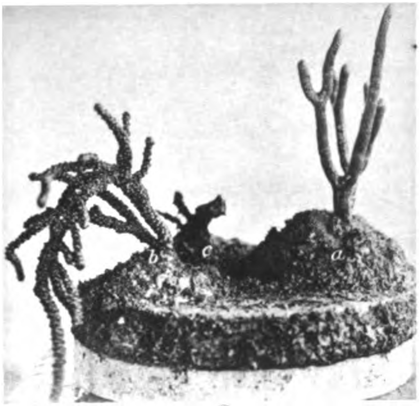

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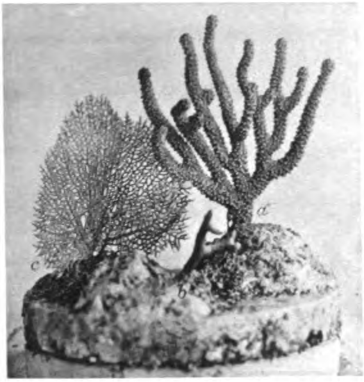

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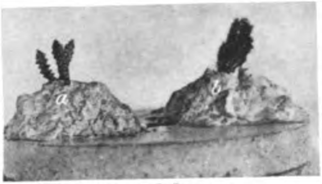

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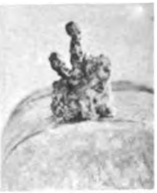

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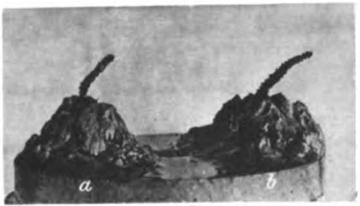

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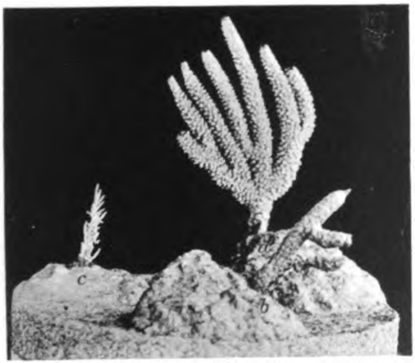

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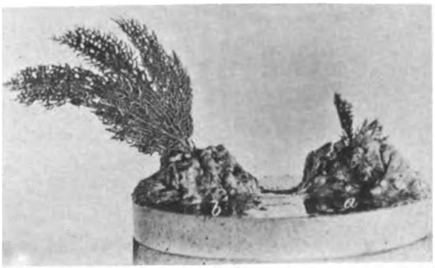

14

VII.

GROWTH-CHANGES IN BRITTLE STARS.

BY HUBERT LYMAN CLARK.

Three plates.

GROWTH-CHANGES IN BRITTLE-STARS.

By Hubert Lyman Clark.

INTRODUCTION.

Although the systematic work of Ljungman, Lütken, Lyman, and Kœhler, which has brought to light hundreds of species of brittle-stars, has been of a very high quality, the real interrelationships of the genera and families within the group are still virtually unknown. Indeed the definitions of most of the families and of many of the genera are so hazy as to be exasperating, and the more one studies the system at present in use the more assured one becomes that it is artificial and unnatural. The first step towards a rational classification was that taken by Bell (1892), when he divided Recent ophiurans into three orders, based on the character of the arm-vertebræ. Unfortunately his subdivision of these orders is not so satisfactory, yet no one has attempted any essential improvement on it. Whether the orders proposed by Bell represent natural groups which have a definite place in the phylogenetic history of the brittle-stars has not yet been critically determined; we can only say that they seem to be such.

Although the chief reason why our classification of the brittle-stars is so unsatisfactory is undoubtedly because no sustained attention has been given to the problems involved, there are subsidiary reasons which should not be overlooked. One of these is that the class shows a rather remarkable morphological homogeneity so that the differential characters which undoubtedly exist are very considerably overshadowed by the extraordinary development of species in the group, and as a consequence the systematic work hitherto done has been almost exclusively concerned with the description of new forms.

A second reason why so little progress has been made towards the natural classification of the group is because the development of so few species is known and in nearly all the known cases attention has been centered on the larval structures or on the metamorphosis. Until very recently the wealth of important data to be found in post-larval stages has been almost wholly overlooked, largely, no doubt, because suitable material is not easily obtained. But Ludwig (1899) and, more recently, Mortensen (1912) have made notable contributions to our knowledge of these most interesting stages.

A third reason why our progress has been so slow is the comparatively small amount of paleontological evidence available, together with the tendency of zoologists to ignore such evidence as there is. Recent work by the Sollases (1912) is an encouraging step along the right lines.

Finally, no attention whatever has been paid to localized stages, the importance of which, in both plants and animals, was pointed out by Jackson (1899) some years ago. They are remarkably well shown in the ophiuran arm, and the attempt is made in the present paper to point out some of them; the chief difficulty is our lack of knowledge, save in a very few species, of the ontogenetic stages with which to compare them.

Brittle-stars are conspicuously common on the reefs and shores of Jamaica, and when I was invited to make use of the marine laboratory of the Carnegie Institution of Washington, located, during February and March 1912, at Montego Bay, Jamaica, I very gladly availed myself of the opportunity, determined to secure, if possible, material which would throw light on the post-larval development of some brittle-star.

I wish to express here my thanks to the authorities of the Museum of Comparative Zoölogy for the necessary leave of absence from my duties there.

Material was easily secured at Montego Bay and young stages were obtained of three different brittle-stars. The purpose of the present paper is to report the results of my studies on these three species, to sum up our present knowledge of the post-larval development of brittle-stars, and to suggest a few conclusions that may be drawn from this knowledge which will be of service in future work.

MATERIAL.

Although species of *Ophioderma* and of *Ophiocoma* are the most common brittle-stars at Montego Bay, no really youthful stages of species of these genera were found. As *Ophiocoma* is known to breed in the early summer, and *Ophioderma* is also a summer breeder, at least farther north, it is quite probable that the season of the year was the reason why the young of these genera were not found. This was to be regretted, since these genera represent widely different families and in neither is there any species whose development is known beyond the metamorphosis. Grave (1900) has worked out the early development of *Ophioderma brevispina* from material collected near Woods Hole, Massachusetts, and has published a preliminary account (1898) of the early stages in *Ophiocoma echinata*. In neither case, however, were post-larval stages available, and I had therefore hoped it might be possible to supplement Grave's work from material obtained at Montego Bay. Although disappointed in this respect, I had the good fortune to find that in the seaweed and fixed animal life (sponges, hydroids, ascidians, etc.) which form great masses on the mangrove roots, at the Bogue Islands, west of Montego Bay, brittle-stars literally swarmed. Of these the commonest was *Ophiactis savignyi* (Müller and Troschel), which was exceedingly abundant in the cavities of a red sponge, individuals of all sizes, from young with disk barely a millimeter across up to adults whose disk exceeded 5 mm., occurring by the hundred. In the same sponge there also lived, although not in anything like the same abundance, the widely distributed and well-known *Amphipholis squamata* (Delle Chiaje), whose early development and metamorphosis have been studied by both Fewkes (1887) and Ludwig (1881). I secured a good series of this species and, owing to its viviparous habit, was able to get some very young material.

The third species of brittle-star of which I secured young also inhabited this red sponge and the adults and half-grown specimens were very abundant. It is *Ophiothrix angulata* (Say), a well-known West Indian species of a cosmopolitan genus, particularly interesting because it is the type genus of one of the very few well-characterized families of brittle-stars. Apparently *Ophiothrix* was not breeding, as *Ophiactis* and *Amphipholis* appeared to be, and the young were very scarce. But enough were obtained to throw considerable light on the post-larval development of the species.

METHODS.

When the abundance of *Ophiactis* in the red sponge was discovered, it was hoped that they could be kept in aquaria and studied as living material, but this proved to be impracticable. If they were removed from the sponge and put in clean water by themselves, they soon became sluggish and died in a comparatively short time; few lived more than 24 hours. On the other hand, if pieces of the sponge were placed in aquaria, the water became foul with astonishing rapidity and the death of all animals speedily ensued. The observations made on the brittle-stars in life were therefore of little importance. Material was preserved for further study by the very simple method of killing with alcohol, or with formalin in which corrosive-sublimate was dissolved, after the animals had been narcotized with magnesium sulphate (Mayer's method). The best material proved to be that which had been killed and preserved in alcohol. As the calcareous plates were the parts particularly desired for study, of course any acid reagent was out of the question. Work on the preserved material has been greatly facilitated by the use of sodium hypochlorite. This alkaline reagent is so powerful a solvent of organic matter that when used in full strength it will reduce a small brittle-star to a heap of calcareous particles in a few minutes. But as it may be diluted with water and mixes readily with glycerine without affecting the "clearing" properties of the latter, it can be perfectly controlled, and the solution of the organic matter and the separation of the calcareous plates can thus be accomplished as rapidly or as slowly as one wishes. Usually glycerine served as a clearing agent, but occasionally better results were obtained from the use of xylol. The latter, however, will not mix with the hypochlorite.

Attention has been centered on the skeleton, since it is well-known that in all echinoderms, except holothurians, modifications of the skeleton almost always accompany changes in any of the soft parts. This is particularly true apparently of ophiurans, the only soft parts which show any evident modification without corresponding skeletal changes being the podia (tube-feet), and even they show relatively little diversity.

The investigations have been strictly confined to growth-changes, as indicative of relationships within the class. I have intentionally avoided, on the one hand, any discussion of *histological* changes, partly because this was aside from the ends in view and partly because such changes have been so ably and fully described for *Ophiactis* by Simroth (1876). On the other hand, I have not entered into any discussion of the homologies of the skeleton in ophiurans as compared with starfishes, nor of the homologies of the jaws, as compared with the arms, since Ludwig's (1878, 1879, 1880, 1881) work has rendered any such contribution on my part quite superfluous. Suffice it to say that in all of my work I have had the homologies suggested by our Nestor of echinoderm morphology constantly in mind, and I have seen nothing whatever to cast any doubt upon his interpretations of ophiuran structure, with the possible exception of the homology of the peristomal plates. On this question I do not care at present to express an opinion.

RESULTS.

The facts which I have been able to ascertain will undoubtedly be more plainly set forth if each of the three species is treated by itself. *Ophiactis* and *Amphipholis* are unfortunately rather closely related to each other, the morphological differences between them being of little importance. *Ophiothrix*, on the contrary, is quite different and shows some interesting peculiarities. As *Ophiactis* seems to be undoubtedly less specialized than *Amphipholis*, it may well be the first genus discussed.

OPHIACTIS SAVIGNYI (Müller and Troschel).

The large number of localities from which this species has been recorded, led me to give special attention to the identification of the specimens from Montego Bay. I have compared them with specimens, most of them identified by Lyman, from Zanzibar, Mauritius, Singapore, the Philippine Islands, the Pelew Islands, Fiji, Hawaii, Lower California, Panama, Florida, the Bahamas, the Bermudas, and Brazil. Absolutely indistinguishable in every essential particular, these numerous specimens prove that this species is truly tropicopolitan. It is one of the small species of the genus, the disk-diameter of adults being usually about 5 mm. and very rarely exceeding 7 mm. There are usually 6 arms, but about one specimen in ten is perfectly pentamerous. In some lots the proportion of pentamerous specimens runs considerably higher than this, while in others the hexamerous symmetry is almost unvaried. I have failed to find any correlation between the pentamerous symmetry and any other character of the individual, nor is it any more frequent in adult specimens than in the young. In addition to its hexamerous symmetry and small size, *savignyi* is characterized by its color (above green, more or less variegated with whitish or yellowish; below whitish or yellowish), its rather short arms (5 to 6 times the disk-diameter), long and large radial shields, spinelets on disk especially around the margin, 5 or 6 arm-spines, and 2 (sometimes 1 or 3) oral papillæ. The genital slits are well-developed and bursæ seem to be present, so that in these particulars as well as in the presence of conspicuous radial shields, *savignyi* is easily distinguished from the European *Ophiactis virens*. Like *virens*, however, *savignyi* is characterized by the remarkable extent to which schizogony is carried as a means of reproduction. The process is apparently identical in the two species and has been very fully described by Simroth (1876), whose observations were based on the European form. The process seems to begin, in *savignyi* at least, almost as soon as the adult form is assumed, for the youngest specimen found has already divided at least once (pl. 1, fig. 2).

No evidence was secured on the important points of egg-laying and larval form in *Ophiactis*. None of the adults contained either embryos or eggs large enough to be visible under a lens. The impression gained from the examination of the living material was that the eggs are laid and fertilization and development take place outside the body of the mother. There was no indication whatever that the species is viviparous. It was not possible to decide whether egg-laying was completed for the season by March first, but the appearance of the adults and the great scarcity of very young specimens led me to the opinion that breeding was over.

My observations confirm Simroth in his statements that there is no special plane of division in schizogony, and that the process shows more or less individual diversity. Thus in fifteen young specimens selected at random, showing evidence that division had recently occurred, fourteen had 3 arms, the other only 2; the latter had 3 jaws and 3 oral shields, however. Of the fourteen 3-armed specimens three have 4 jaws and four have only 2. The plane of division, which passes through two opposite interradii, does not then divide a jaw, but passes sometimes on one side and sometimes on the other. Only about half the time does it divide the animal into perfectly complemental halves. The process of healing and the simultaneous rapid growth of the water-vascular system have been well described by Simroth. Almost, if not actually, before the circular canal is completely healed buds which will give rise to new radial canals have pushed out, one at each side of the healing margin of the now semi-circular disk, and before there is any external evidence of the new half-disk the buds of two new arms, one at each side, become visible. Before their growth has proceeded very far, however, a third arm appears between them, associated with the third outgrowth of the now completely healed circular canal. The failure of this third radial canal to start development probably accounts for some of the 5-armed individuals met with, but as the plane of division occasionally leaves 4 arms on one disk-half and only 2 on the other, it is evident that these 2-armed halves will form 5-armed adults even with a normal development of 3 new radial canals. As no 4-armed or 7-armed specimens were seen (excepting of course the 4-armed complements of 2-armed individuals), it would seem to be true that the presence of 4 arms on a regenerating half always prevents the *growth* of the third new radial canal, while the presence of only 2 arms always prevents the *suppression* of the third new arm. In very young regenerating individuals, the difference in size between the third new arm and its two fellows is usually quite marked (pl. 1, fig. 1).

As Simroth, Fewkes, and Ludwig have all pointed out, the development of the skeletal pieces of the new arms takes place rapidly at the tip of the growing radial canal, forming a covering about it and, as it gives rise to pairs of podia, forming the vertebræ and arm-plates of successive joints. In *Ophiactis* the podia are not peculiar in any way, but are simple, slightly tapering, blunt tubes with thin, smooth walls. We may therefore turn our attention to the skeletal plates.

DISK-COVERING.

In the youngest specimen found the disk measured a millimeter across, but it was only semi-circular, since division had already occurred at least once (pl. 1, fig. 2). That not more than one division had occurred is indicated by the arrangement of the plates on the disk. The older half of the animal is easily distinguished from the new one developing, the size of the plates in the two areas contrasting sharply. The older half is covered by 3 pairs of radial shields, a central plate, 3 radial plates, and 6 interradials. On the developing half-disk it is less easy to identify the plates, but there seem to be 3 pairs of radial shields, separated from each other by interradial plates. The arrangement of the plates on the older half corresponds well with that which Ludwig (1899) figures for specimens of *Ophiactis asperula* with the disk only 0.68 to 0.87 mm. across. The only difference is in the relative sizes of the different plates; in *asperula* the central and radial plates are conspicuously bigger than the radial shields, but in *savignyi* this is not so.

There seems no reason for doubting that in its earliest stages the disk-covering of *savignyi* is identical with that of *asperula*, and Ludwig (1899) has shown that the same is true of *Ophiactis kröyeri*. In *savignyi*, however, the occurrence of schizogonous reproduction in very early life interferes with the orderly and uniform development of the disk-covering, shown by *asperula* and *kröyeri*, so that after division has occurred twice it is difficult, if not impossible, to distinguish the primary plates. This is well shown by figure 1 of plate 1, which represents a specimen with a disk rather more than 1 mm. in diameter. Division has apparently occurred twice and it will be seen how irregular is the resulting disk-covering. The irregularity seems to be due to the fact that whereas, in *asperula* and *kröyeri*, the new plates are formed near the distal margins of the radial and interradial plates, in the regenerating *savignyi* they seem to be formed at the proximal edge of marginal plates. In other words, the typical disk-covering develops centrifugally, while the covering of the regenerated disk arises centripetally. As division appears to take place repeatedly, by the time *savignyi* is full grown there is not the least trace of the original primary plates nor of any very definite arrangement in the numerous small plates covering the center of the disk. The radial shields are always conspicuous and cover a large proportion of the disk. Soon after the disk is a millimeter across, spinelets arise in connection with the marginal disk-plates. These spinelets are not outgrowths of the plates, but arise from separate centers of calcification in the tissue covering their outer surface. Not often is more than one associated with a single plate, and many plates, especially near the center of the disk, have no spinelets. When fully formed the spinelets are attached to the underlying plates, but no actual union takes place and the spinelets are always movable to some extent. The number of these spinelets varies in different individuals, but they seem to be relatively most numerous in young specimens. Adult, dry specimens commonly show them plainly, but they are often difficult to detect in fresh or alcoholic adults.

MOUTH-PARTS.

As has been stated above, the plane of division in schizogony does not cut through the jaw, but passes to one side, so that the resulting animals may have either 2, 3, or 4 jaws. The formation of new jaws takes place rapidly, but follows of course the formation of the new rays. Consequently the same factors which control the new-ray formation govern the development of new jaws. In case there are, after fission, only 2 jaws on one resulting half (pl. 1, fig. 3), the outer side of each of the lateral rays must assist in the formation of new jaws, while in case there are 4 jaws each of the new-formed lateral rays will give rise to jaw-elements only on that side which is next the youngest ray. The formation of the jaws takes place as already described by Ludwig (1881) for *Amphipholis* and I have nothing of importance to add to his account. Ludwig has not discussed, however, the formation of teeth and oral papillæ, nor did Fewkes (1887) pay any special attention to these points. I found it possible to trace the development of teeth and oral papillæ in both *Ophiactis* and *Amphipholis*, but as my material was more satisfactory in the latter genus, the account may for the present be deferred. I need only say here that there is no difference between the two genera save in the number and form of the teeth and papillæ. As is well known, the torus (the plate which bears the teeth) forms at the tip of the jaw where the two jaw-plates (adambulacrals) meet. It is at first about as wide as high, but grows rapidly vertically and soon is twice as high as wide or even more. As will be shown under *Amphipholis*, the oldest tooth is at the top of the torus, the new ones forming below it. As soon as a tooth is well formed, the part of the torus against which it rests is resorbed and thus a socket is formed for it. The process of resorption goes so far that the torus becomes perforated (pl. 1, fig. 5). On an adult jaw of *Ophiactis savignyi* there are 5 or 6 teeth of approximately uniform size (pl. 1

fig. 4), but on a growing jaw the youngest tooth, the lowest, is distinctly the smallest.

ARM-BONES OR VERTEBRÆ.

Although Simroth (1876) has figured the vertebræ of *Ophiactis virens*, it has seemed desirable to give a series of illustrations of the vertebræ in *savignyi*, not merely because they are obviously different from those of *virens*, as shown by Simroth, but because the series in each arm, taken as a whole, reveals stages in development which are of particular interest. As the origin and growth of the vertebræ are in all essentials exactly as Ludwig and Fewkes have described in *Amphipholis*, there is no occasion to go into great detail here. The growing tip of the radial water-tube is protected by a cylindrical plate or, better, a calcareous cylinder, back (proximal) of which arise all the calcareous structures of the arm. The first plates to appear are the side arm-plates, but no sooner are they well under way than the rudiment of the under arm-plate appears followed by the pair of calcareous rods which are to form the vertebra, lying side by side, above (dorsal) the water-tube. They develop very rapidly and soon the proximal ends are in contact with each other (pl. 1, fig. 6). New joints are arising distal to them, and by the time half a dozen joints have appeared the two halves of the vertebra are in very close contact; when 10 joints have formed it will be found that the first-formed vertebra is now a single, well-formed, bilateral structure, which can hardly be separated into its component halves. At this time the vertebra is much longer than wide, a little wider than high, and narrower distally than proximally. It soon begins to broaden at the distal end, a sort of knob or projection forming on each side (pl. 1, fig. 8).

Further development is chiefly along three lines: rapidly increased proportional breadth, greatly increased height, and growth of spurs, knobs, ridges, and hollows. The increased breadth is easily seen by comparing the vertebra of the thirtieth segment of an adult arm (pl. 1, fig. 11) with that of the fifty-fifth (pl. 1, fig. 8); while the length has only increased 25 per cent, the breadth has increased 180 per cent; or compare a fully developed vertebra, as in the tenth segment (pl. 1, fig. 14), with the young one of the fifty-fifth segment, and it will be seen that while it is only twice as long, it is more than six times as wide. The increased height is easily shown in the same way; thus although in segment 10 the vertebra is about 8 per cent higher than long (pl. 1, fig. 18), in segment 55 it is more than three times as long as high. The development of spurs, knobs, ridges, and hollows is equally remarkable and is easily understood by an examination of the figures given. Unfortunately no detailed description of an ophiuran vertebra, with technical names for the different parts, has ever been published, Ludwig, Lyman, and others making use of descriptive phrases for the various parts. Since the structure of the vertebræ will undoubtedly play a more important part in the classification of ophiurans in the future than it has in the past, it is desirable to have a uniform nomenclature for the various parts.

Taking the fully formed vertebra of *Ophiactis* as a type (pl. 1, figs. 14 to 18), we may distinguish the following parts, when the vertebra is seen from above (fig. 14): the *alæ* form the bulk of the adoral end, their upper margins forming the *upper alar ridges;* these alar ridges are widely expanded at their inner end, but do not meet in the mid-line; the space between is of little importance in *Ophiactis*, but is very conspicuous in some genera; it may be called the *zygantrum;* the expanded inner end of each alar ridge is more or less clearly divided into three parts which may be called the *epapophyses;* on each side there is an *aboral, median,* and *adoral epapophysis* and in *Ophiactis* they are all distinguishable, although the aboral is the most conspicuous. Directly in front of the aboral epapophysis is a broad, nearly flat platform, the *protapophysis*, the aboral corners of which are higher than the median portion. Projecting from beneath this median portion may be seen the *zygosphene*, a shining, glassy knob which still shows evidence of its paired origin. At either side, but still lower, are the *aboral hypapophyses;* back of these and about half-way between them and the alæ are the *parapophyses*, not very conspicuous or important ridges closely associated with the median hypapophyses; back of and below the zygantrum is a conspicuous median, unpaired process, the *epanapophysis*, which is particularly noticeable in the vertebræ within the disk (pl. 1, fig. 19); on either side of the epanapophysis, but in a much lower plane, lies a *zygapophysis*.

These are all the parts which are visible from above, but if we look at the adoral surface of the vertebra, (pl. 1, fig. 17) we shall see below the zygapophyses, the adoral pair of hypapophyses, while between the zygapophyses is the important space, the *zygotreme*. Looking at the lower surface of the vertebra (pl. 1, fig. 16) a deep furrow, the *taphrus*, is seen running from between the zygapophyses to the zygosphene; it is bounded on either side by the three hypapophyses and the thin, vertical wall which connects their bases; the taphrus contains the radial water-vessel and nerve; the *lower alar ridges* form the ventral margin of the alæ.

Making use of these terms, then, we may describe the vertebra of *Ophiactis* as short and high, with a small zygantrum, a broad, low protapophysis, a well-marked zygosphene, a large epanopophysis, well-developed zygapophyses, and small, adoral hypapophyses. The basal segments of the arm are so crowded and compressed that they do not fairly show the appearance of a typical vertebra, but from the tenth to the fifteenth segment the vertebræ show clearly their characteristic features. Beyond the twentieth segment youthful characters become evident and these become more and more marked as one approaches the tip of the arm. This is well brought out by comparing a basal joint of a young arm with a corresponding joint of an adult arm. For example, the resemblance is very noticeable between the tenth vertebra (counting from the base of the arm outward) of an arm having 18 joints (pl. 1, fig. 12) and the thirtieth vertebra of an arm with more than 60 joints (pl. 1, fig. 11), but the difference between this young tenth vertebra and the tenth vertebra of the adult arm (pl. 1, fig. 14) is most striking. No doubt the existence of localized stages in the series of vertebræ of the ophiuran arm will be easily demonstrated when the details of arm-structure are worked out for a large number of species and genera.

The development of the young vertebra into the adult deserves a further word. It has already been pointed out that during this development growth longitudinally is very slow as compared with the lateral and vertical growth. Attention may now be given to the important question as to what processes of the adult vertebra are the first to develop and which are the last. In a very young vertebra the alæ are the only evident projections, but in one a little older (pl. 1, fig. 8), the aboral hypapophyses are well marked, the zygosphene is defined, and the aboral end of the protapophysis can be distinguished; there is also an indication of the epanapophysis. In the vertebra of the segment, 5 joints nearer the disk (*i. e.*, the fiftieth), the upper alar ridge is distinct and the protapophysis is fully defined. In the vertebra of the fortieth segment the parapophyses show and the aboral hypapophyses are very prominent. In the vertebra of the thirtieth segment the first indications of the epapophyses are seen in the line of distinction between the protapophysis and the alar ridges. In the vertebra of the twentieth segment this distinction is so far completed that the aboral epapophyses and the zygantrum are well defined.

Before leaving the subject of the vertebræ, a word may be said as to their articulation with each other. The alæ of course furnish the chief surface for muscular attachment, but the parapophyses and hypapophyses are important points of attachment. The zygosphene fits into the zygotreme of the next distal vertebra and is supported on each side by a zygapophysis. The aboral margin of the protapophysis fits into the space below the epanapophysis and above the zygapophyses, and the aboral hypapophyses fit into the space below and outside the adoral pair. So while the zygosphene and zygotreme furnish what might be called the axial joint, which is thus of the ball-and-socket type, too great vertical freedom is prevented by the protapophysis and epanapophysis, while excessive lateral movement is impeded by the aboral and adoral hypapophyses.

ARM-PLATES.

The sequence of formation of the arm-plates has been correctly stated by Ludwig and by Fewkes, their observations on *Amphipholis* being fully confirmed by mine on that genus and on *Ophiactis*. The 2 side arm-plates appear simultaneously, one on each side, directly back of the terminal plate, and are the first indication of the formation of an arm-segment. They very quickly meet in the mid-ventral line and a little later in the mid-dorsal line. They are followed at once by the rudiment of the under arm-plate, which arises directly in front of, or distal to, their ventral line of division. The under arm-plate is soon followed by the upper one, which is formed in a corresponding position on the dorsal side of the arm. In a normally developing young arm, the fourth segment is generally the first one that can be considered complete (pl. 1, fig. 6); the third has only a rudiment of the upper arm-plate; the second lacks the upper arm-plate altogether; the first lacks both upper and under arm-plates. It is true that the rapidity with which new segments are formed shows more or less diversity in different individuals. Apparently, in the regenerating tip of an adult arm the segments are formed more rapidly and develop much more slowly than in a young arm. As a consequence, the sixth, tenth, and fifteenth segments of such an arm are all in about the same stage of development. Simroth (1876) has correctly described how the new "arm-buds" arise on the regenerating half of the disk. As soon as they are visible, the terminal plate appears at the tip just above the water-tube. It grows down and around the tube very rapidly and soon the two sides meet and fuse in the mid-ventral line. By the time this process is complete the first pair of side arm-plates is fully formed (pl. 1, fig. 7). The first side arm-plates meet broadly both above and below, the under and upper plates being relatively very small on the young joints; but both grow more rapidly than the side arm-plates and slowly but surely force the latter apart. By the time 20 segments are formed, the side arm-plates are nearly separated dorsally and the separation is completed before 25 segments have developed. Ventrally the process is a trifle slower, but it is completed before 30 segments are formed. On the basal part of the arm, therefore, the side arm-plates are completely separated above and below, while the under arm-plates are in contact with their fellows and the upper plates with theirs.

As regards their relation to the vertebra which they inclose, in the adult arm-segment it can be easily seen that the side arm-plates are in very close conjunction with the alæ, fitting more or less neatly into the area between the alar ridge and the parapophysis. The under arm-plate is in contact with and apparently somewhat attached to the aboral and median hypapophyses. The upper arm-plate rests on and is perhaps attached to the epapophyses and even to the protapophysis.

ARM-SPINES AND TENTACLE-SCALES.

The first-formed arm-spine is the *lowest* and no additional spine ever appears normally between it and the tentacle-scale. It ordinarily appears as soon as the side arm-plate is well formed, but is usually lacking from the first segment (*i. e.*, the segment directly adoral to the terminal plate). On the second segment the lowest arm-spines are well marked; they each originate from a separate center of calcification close to the aboral margin of the side arm-plate near its lower end. The second arm-spine is the next dorsal to the lowest; it may appear almost simultaneously with the lowest or it may be wholly lacking on the second segment. Although appearing later, the second arm-spines grow more rapidly than those first developed and are soon distinctly larger. The third arm-spine of each vertical series generally appears on the sixth or seventh segment from the tip; it lies just dorsal to the second, of course. The fourth spine, just dorsal to the third, arises very much later; in some individuals it may be found on the fifteenth segment back of the terminal plate, but in other specimens as many as 25 segments may have only 3 spines on each side. In large specimens, a fifth, sixth, seventh, and very rarely an eighth spine appear in each series dorsal to the fourth, but the position of the segments on which these spines first appear differs much in different specimens. In the largest specimen seen, the basal arm-segment has 2 spines, the next 4, the next 5, the next 6, the next 7, and the next 8; then follow 13 joints, each with 7 arm-spines on each side, 23 joints with 6, 24 with 5, and 18 with 4; the rest of the arm is unfortunately missing. The species character (5 or 6 arm-spines) is thus shown from the tenth to the fifty-seventh segment. This is a much more extended series than is shown by the vertebræ, which are typical from about the eighth to the twentieth segment only. The next to the lowest arm-spine is the largest (longest and stoutest), as long as there are only 4, but as the number increases the lower spines grow very little, while the upper ones grow rapidly, so that near the base of the arm the next to the uppermost, or the uppermost, becomes the largest. At the tip of the arm, the spines are smooth, but they soon develop thorn-like projections on the lower, adoral margin, and generally on all sides, especially near the tip. Excepting on the lowest spine, these projections are neither large enough nor sufficiently definitely arranged to have any particular significance, but on the lowest spine they are more characteristic. The lowest spines of the third segment (pl. 3, fig. 12) have an enlarged base and a smooth, nearly cylindrical tip, but those of the sixth (pl. 3, fig. 13) have the thorn-like projections or "teeth" of the lower, adoral margin very conspicuous, especially the terminal one. There are half a dozen or more of these teeth at this stage, but as the spine grows older they increase in number and occupy the other angles of the spine, so that at the middle of the arm the lowest spine (pl. 3, fig. 14), although much more thorny than any of the others, does not differ from them as evidently as it does near the tip of the arm. It can not be said, then, that the lowest arm-spine of *Ophiactis* is, in any sense, characteristic.

The tentacle-scales in *Ophiactis savignyi* are large and conspicuous, although there is but one on each pore. It arises almost as soon as the lowest arm-spine, but it is associated with the ventral surface of the side arm-plate. It arises from a separate center of calcification and its rudiment appears simultaneously with that of the under arm-plate. There is nothing in the development of the tentacle-scales to determine whether they are homologous with the arm-spines or not.

SUMMARY OF GROWTH CHANGES.

1. Fission occurs as soon as adult form is assumed; it usually results in complemental halves, but may leave *four* rays on one half and only *two* on the other.
2. The 5-rayed individuals arise in either one of two ways: by the formation of a normal 3-rayed half on a regenerating 2-rayed individual,

or by the failure of the median ray to develop on the regenerating side of a normal 3-rayed individual. But only about one specimen in ten has 5 rays.

3. In fission the dividing plane does not cut through a jaw, but passes on one side or the other; accordingly a half-disk may have 2, 3, or 4 jaws; normally there are 3.
4. The disk-covering, when the adult form is first assumed, consists of the typical primary-plates, shown by Ludwig to be characteristic of *Ophiactis*, but owing to repeated fission, the covering of the disk is ultimately made up (aside from the large radial shields) of small plates with no regular arrangement.
5. The torus bears at first none, then 1, 2, 3, 4, 5, and sometimes 6 teeth, of which the oldest (first-formed) is uppermost and the youngest is lowest (most ventral).
6. Young vertebræ are very much longer than high or wide; the alæ are the first outgrowths to appear, soon followed by the aboral hypapophyses; the zygosphene, protapophysis, and epanapophysis appear almost immediately thereafter.
7. Typical vertebræ occur in the adult arm from about the eighth to the twentieth segment.
8. Of the plates covering the vertebræ, the side arm-plates appear first, followed by the under arm-plates and lastly by the upper. The side arm-plates, of any one segment, are broadly in contact with each other both above and below on the young joints, but are ultimately separated dorsally by the growth of the upper arm-plates and ventrally by the lower.
9. The lowest arm-spine is the first formed; all succeeding spines arise serially dorsal to it.
10. The typical number of arm-spines is to be found on about the tenth segment of an adult arm and thence distally for a varying number of segments, rarely as many as 40.
11. The lowest arm-spine has no characteristic form.
12. The tentacle-scales appear with the first under arm-plate; they may not be homologous with the arm-spines.

AMPHIPHOLIS SQUAMATA (Delle Chiaje).

The peculiar habitat of the specimens of *Amphipholis* collected at Montego Bay led me to question whether the species was really identical with that found on shelly bottoms in New England waters. But critical comparison of these Jamaican specimens with others from Woods Hole (Massachusetts), Casco Bay (Maine), Grand Manan (New Brunswick), Norway, and Naples has forced me to the conclusion that they can not be distinguished and are undoubtedly entitled to the name *squamata*. At Montego Bay, the species was found only in the red sponge in which *Ophiactis* occurred so abundantly, but in that situation it was fairly common. The smallest free-living specimen seen had the disk a millimeter across and of an orange-red color, a peculiarity of the young noted by Fewkes. Most of the specimens with disks more than 2 mm. across contained young in a more or less advanced stage of development. The youngest embryo examined had the disk 0.35 mm. across, and the arms, each with 3 segments, projected only 0.20 mm. beyond the disk margin. The largest adult did not exceed 3 mm. in diameter.

In two particulars the *Amphipholis* from Montego Bay showed rather striking differences from those studied at Newport by Fewkes (1887), who says that 4- and 6-armed young "were repeatedly found" and that "adults with 6 arms were common." I have not found a specimen in my Jamaican material, either adult or young, which varies in any way from the typical pentamerous condition. Again, Fewkes says that "ordinarily a gravid adult would have from 10 to 15 (generally 10) free young." In the Jamaican form the number of young ranged from 1 to 3, but in no case were more than 3 seen. There were no 2 of the 3 at the same stage of development; for example, in one typical case one young was 0.50 mm. across with 5-jointed arms, a second was 0.63 mm. across with 8-jointed arms, and a third was 0.80 mm. across with 11-jointed arms. The young are not born until the disk is nearly a millimeter across; one mother only 2.2 mm. across the disk contained a young one 0.90 mm. across. Fewkes does not mention the size of his adults, and it is quite possible that they were much larger than the Montego Bay specimens. If so, that fact might account for the physiological differences just noted, in the frequency of variation, and the number of young. Or it is possible that the small number of young may have been due to the breeding-season being nearly over at Montego Bay. No special attempt was made to settle the matter, as it was not until after my return to Cambridge that I had any doubts as to the breeding-season being at its height.

Unlike *Ophiactis*, *Amphipholis* shows no evidence of schizogonous reproduction, but ordinary reproduction by ova begins very early in life. The development has been so carefully worked out by Fewkes (1887) and Ludwig (1881), as well as by several other students, that there is little that is new for me to set forth, except in certain details omitted by these earlier writers. As in the case of *Ophiactis*, attention has been centered on the skeletal parts, as neither the podia nor any other soft parts reveal any features of interest, from the present point of view.

DISK-COVERING.

In the youngest specimens seen the disk was covered by 16 plates (a central, 5 large radials, and 10 interradials) as figured by Ludwig, but the 5 pairs of radial shields were already beginning to appear. By the time the disk is 0.80 mm. across, there are 5 more radials and 5 more interradials, while the radial shields are now easily recognized as such. The new radial and interradial plates arise between the central and the primary plates of radii and interradii. With increasing size of disk the conspicuousness of the primary plates disappears, and while they can sometimes be made out in the adult, they are not as a rule easily seen. The adult disk is covered by 150 to 300 plates. No spinelets develop in connection with any disk-scales.

MOUTH-PARTS.

My observations on the mouth-parts confirm fully the work of Fewkes and Ludwig. I am, however, able to add certain details in regard to the teeth and oral papillæ which were either overlooked by those writers or considered of too little importance to note. As mentioned under *Ophiactis*, the torus forms at the tip of the jaw as a small plate about as wide as high, but rapidly becomes vertically elongated. Before it is twice as high as long, the first tooth is formed from a triradiate spicule lying close to the torus on its inner side, near its upper end. This spicule rapidly forms a triangular pyramid, the base of which rests very closely against the torus (pl. 2, fig. 5). Fewkes says that "the teeth are not separate centres of calcification," but appear "to grow out directly from the adoral region of the torus." My first impressions were that this statement was correct, so closely are the teeth associated with the torus, but careful maceration with sodium hypochlorite made it possible to determine the truth. So closely does the young tooth press against the torus, that resorption (as stated under *Ophiactis*) takes place in the latter and a socket is formed in which the tooth sets very snugly, but probably not immovably. The first-formed tooth attains some size (pl. 2, fig. 6) before the second is formed. As the torus elongates vertically the second tooth arises below the first and subsequently a third forms beneath that, and finally a fourth below that; the maximum number in *Amphipholis* from Montego Bay seems to be 4; probably larger specimens will be found to have 5. The growth of the torus seems to be chiefly at the lower end, and that of the teeth is mainly distal. In the adult torus the sockets, in which the teeth are set, completely perforate its skeletal tissue, with the occasional or perhaps usual exception of the lowest. The torus is widest and thickest at the top.

Fewkes has laid considerable stress on the formation of a spinous projection at the distal end of the second adambulacral plate, during the formation of the jaw, which he considers homologous with an arm-spine. There is no doubt of the existence of this process. Ludwig and others refer to it and I have had no difficulty in finding it (pl. 2, fig. 1). But in following its development we find it never becomes a spine or any other special organ; it simply forms the distal, concealed end of the adoral plate, to which the second adambulacral plate gives rise. We have already seen under *Ophiactis* that the arm-spines arise from separate centers of calcification, but neither Fewkes nor Ludwig noted this fact. Had it been observed by Fewkes, he could hardly have homologized the distal process of the adoral plate with an arm-spine; he seems to have been misled by a superficial resemblance and to have overlooked the important fact that spines do not arise by budding or breaking off from previously formed plates.

The origin and development of the oral papillæ in *Amphipholis* is of especial importance, since their appearance in the adult is the character upon which the genus is based. They arise very early in the development of the jaw, for while the adambulacral plates which form the latter are still quite distinct, their rudimentary beginnings may be seen (pl. 2, fig. 2). At this stage there are two papillæ on each side of each jaw; one is at the outer (adradial) proximal corner of the first adambulacral, while the other is on the inner (proximal) side of the second adambulacral, just distal to the oral tentacle. Later a third papilla arises on the adradial side of the first adambulacral, close behind the first papilla. The time of appearance of this third papilla shows considerable diversity; usually it forms very soon after the other two, but occasionally it is delayed, and even a specimen a millimeter across may lack it (pl. 2, fig. 3). The typical generic character (pl. 2, fig. 4) is commonly recognizable by the time the disk is 1.30 mm. across. The existence of the stage with only the proximal and distal papillæ (pl. 2, figs. 2 and 3) is of extraordinary interest, for that is the condition characteristic of the genus *Amphiura* and we are therefore justified in saying that *Amphipholis* passes through an *Amphiura* stage. Evidently light is thus thrown on the phylogeny of *Amphipholis*. The appearance of the rudimentary oral papillæ and their relation to the first and second adambulacral plates, with their accompanying podia, suggest the possibility that the oral papillæ are homologous with tentacle-scales. Investigations on other genera will be necessary in order to determine the point.

ARM-BONES OR VERTEBRÆ.

Ludwig (1881) has worked out so completely the development of the vertebræ that there is really very little to be added. My observations do not disagree with his in a single essential feature, although in details of secondary hollows and ridges my impressions of an adult vertebra do not agree with his figures. The largest vertebra I have examined is relatively longer than his largest, but, as I have already stated, the Montego Bay specimens of *Amphipholis* were all small, and their arm-segments are undoubtedly relatively longer than those of larger specimens. The adult vertebra may be described as somewhat elongated, with a small zygantrum, a low, long protapophysis, a well-developed zygosphene, a large epanapophysis, well-developed zygapophyses, and very small adoral hypapophyses. In all essentials it is like that of *Ophiactis*, but relatively much longer and somewhat lower; the protapophysis is narrower, the parapophyses more conspicuous, and the epapophyses less well marked. In all these particulars the vertebra of *Amphipholis* is more youthful than that of *Ophiactis*.

ARM-PLATES.

In no particulars does the development of the arm-plates differ from what has been shown for *Ophiactis*. The side arm-plates appear first and are followed by the under arm-plate, while the upper arm-plate appears last of all. In one point, however, development does not go so far in *Amphipholis* as in *Ophiactis*. The side arm-plates are never fully separated, either above or below; at least, in the largest specimen I have examined, one which has 40 arm-segments, the side arm-plates are still distinctly in contact even in the first basal segment. In this particular character, then, *Amphipholis* is more primitive or youthful than *Ophiactis*.

ARM-SPINES AND TENTACLE-SCALES.

One of the most peculiar features of the development of *Amphipholis* is the fact that it is not the lowest arm-spine which appears first, but the next to the lowest. So unwilling was I to believe this that it was only after repeated observations, on many specimens, that I satisfied myself there is no doubt about the fact. Development at the tip of the arm is so rapid that each side arm-plate of the newest segment usually bears two spines before a new segment begins to form; this first segment may also have the rudiment of an under arm-plate. If one examines this distalmost segment when the side arm-plates have just met in the midventral line, the rudiment of an arm-spine will be found on the aboral margin of each side arm-plate. Almost immediately a second rudiment appears *below* (ventral to) the first, so that there are two rudimentary spines on each side. They remain of about equal length for some time, but the upper is the stouter and more thorny (pl. 3, fig. 11). On the third segment they are of about equal length, but on the fourth the lower is longer. By the time 8 segments are formed a third spine appears on each side arm-plate above the other two. This rapidly becomes the longest of the three, while the middle one is the stoutest and roughest. By the time 25 segments are complete a fourth spine dorsal to the third may arise, but it does not become noticeably longer than the third and is generally shorter. The development of the "second" spine before the lowest is so contrary to *a priori* expectations that some explanation is necessary. It is true that in its early appearance (pl. 3, fig. 11), it resembles the lowest arm-spine of *Ophiactis* (pl. 3, fig. 13) and it is possible to consider the two homologous, since each is the first spine formed on the segment. But in that case the *lowest* arm-spine of *Amphipholis* requires explanation, for there is nothing homologous to it in *Ophiactis*. A preferable explanation seems to me to be the suggestion that we have here an extraordinary case of acceleration in development. We have already seen in *Ophiactis* that after the "second" spine is formed it grows more rapidly than the lowest and soon exceeds it in size. Now, the whole appearance of young *Amphipholis* shows that its development within the mother's body has led to an acceleration which is most noticeable at the tips of the arms, although marked on the disk. Is it not possible that acceleration in the development of the arm-spines has led the more-rapidly growing "second" spine to appear before the lowest? Such a suggestion seems to me well within the bounds of possibility. In any case, however, neither the lowest nor the second arm-spine develops any unusual hooks or teeth or assumes any particularly characteristic form.

Although the basal joints of the arm show two tentacle-scales on each side, one associated with the under arm-plate and one with the side arm-plate, it is a remarkable fact that at the tip of the arm there are no tentacle-scales at all. It is not until a dozen or more segments are formed that the first tentacle-scale appears and it is associated with the side arm-plate, arising near its outer margin, at the base, from a separate center of calcification. The origin and appearance suggest homology with the arm-spines. Almost immediately after the formation of the first tentacle-scale, a second one associated with the under arm-plate begins to form and is usually visible after one or two segments have given rise to the first one. The exact place of appearance of the tentacle-scales is subject to a good deal of individual variation; thus in a specimen with 21 or 22 segments in each arm, the terminal 15 or 16 have no tentacle-scales, but the sixteenth or seventeenth has one associated with the base of the side arm-plate, while the second scale may be seen on the eighteenth or nineteenth segment in 4 arms, but not until the twentieth in the fifth; on the other hand, in older specimens, with 30 or 40 arm-joints, the first tentacle-scale may appear only 12 segments from the tip of the arm and the second on the fourteenth. The first tentacle-scale has every appearance of being the homologue of an arm-spine, but the second is apparently quite a different organ. It must be considered as a possibility that this *second* tentacle-scale in *Amphipholis* is homologous with the single tentacle-scale of *Ophiactis*, but the question involves the examination and study of many related genera before it can be answered.

SUMMARY OF GROWTH CHANGES IN AMPHIPHOLIS SQUAMATA.

1. The disk-covering when the adult form is first assumed consists of the typical primary plates, figured by Fewkes and by Ludwig. Rapid growth, however, soon decreases the conspicuousness of these plates and in the adult they are seldom recognizable.
2. The torus of the adult bears 4 teeth, which do not arise as outgrowths from it, but originate from separate centers of calcification. The uppermost tooth is oldest, the lowest youngest.
3. The second adambulacral plate, which becomes the adoral plate in the adult, does not bear a spine at its distal end at any stage of its development, nor does a spine arise at any time by a separation of a projection of the original plate.
4. The oral papillæ arise from separate centers of calcification associated with the first two adambulacral plates. They may be homologous with tentacle-scales.
5. At an early stage of development, *Amphipholis* has only 2 oral papillæ on each side of the jaw, as in *Amphiura*. It may therefore be said to pass through an *Amphiura* stage.
6. The vertebræ of *Amphipholis* are essentially like those of *Ophiactis*, but have retained certain youthful features, greater relative length, less relative height, narrower protapophysis, more conspicuous parapophyses, and less well-marked epapophyses.
7. Arm-plates arise in the same sequence as in *Ophiactis*, but the side arm-plates of any one segment are never separated from each other, either above or below. The arm is thus more youthful than that of *Ophiactis*.

8. The next to the lowest arm-spine and not the lowest is the first one formed, but the first segment usually has both, on each side, before the second segment arises.
9. The lowest arm-spine never develops any special or characteristic form.
10. The distal arm-segments have no tentacle-scales, but at the base of the arm each pore has two; one, associated with the side arm-plate, arises after 12 to 18 segments have been formed; the other, associated with the under arm-plate, arises one or two segments later. The former may be homologous with an arm-spine; the latter may be homologous with the tentacle-scale of *Ophiactis*.

OPHIOTHRIX ANGULATA (Say).

Two species of *Ophiothrix*, *angulata* and *œrstedii*, are very common in shallow water throughout the West Indian region. Both are exceedingly variable in coloration and the specific differences between them are often hard to make out. The commoner and more variable species is *angulata*, and at Montego Bay a dozen specimens could be gathered to every one of *œrstedii*. The latter has very characteristic lines of white or cream-color cross-banding the upper surface of the arms, and these are rarely, if ever, lacking, though they are occasionally faint. In *angulata* such cross-lines are rarely indicated, but there is present a longitudinal stripe of white, or some light shade, running the length of the arm. Although such a distinction seems trivial, especially when the rest of the coloration is very variable, it is really remarkably constant and specimens can generally be assigned to one species or the other at a glance.

As is generally the case when a species is very common, *angulata* occurs in very diverse habitats. It is quite common at Montego Bay among coral fragments on the reefs, it is very common among the eel-grass on the flats, and it is exceedingly common among the bryozoa, sponges, mollusks, hydroids, and ascidians growing in masses on the mangrove roots among the Bogue Islands. Most of the young specimens obtained were found in the last-named habitat, especially in the cavities of the same red sponge in which *Ophiactis* and *Amphipholis* occur. No very young specimens were found, the smallest having the disk 2 mm. across. While this is greatly to be regretted, much interesting information has been secured from the young ones that were obtained. No indication of schizogony nor of variation from the typical pentamerous form was seen.

COLORATION.

Neither *Ophiactis* nor *Amphipholis* has a color pattern sufficiently distinctive to make its development a matter of any significance, but as already stated, *Ophiothrix angulata* is most easily distinguished from its nearest ally by a characteristic color pattern. The development of this pattern, therefore, is of no little interest. The youngest joints at the tip of the arm are colorless and contain no pigment. In the third, fourth, or fifth segment, however, a little pigment begins to appear and gives a tinge of color, deepening with the increasing age of each successive segment. This pigment is bright purple of a deep shade. It first appears in the side arm-plates near their upper ends and gradually spreads distally and to some extent ventrally. It soon invades the margins of the upper arm-plates and follows them to their distal end. In a typical case it is very sharply confined to the sides of the upper arm-plates and fails to cross from one side arm-plate to its fellow of the same segment. Consequently there is an unbroken, unpigmented (*i. e.*, apparently white) stripe along the dorsal surface of the arm. In some specimens pigment fails to invade the distal corners of the upper arm-plates and thus there appears to be a row of white spots along each side of the stripe; or the pigment may fail in the spine-bearing ridge of the side arm-plates and then the dorsal white area of the arms is markedly increased. On the lower side of the arms the pigment also spreads longitudinally in the side arm-plates and in the margins of the lower arm-plates, but it rarely if ever crosses the latter. In other specimens the pigment spreading in the margin of the upper arm-plates follows back along the converging sides to the proximal end and there is confluent with its fellow of the other side and with the pigment of the side arm-plates. In such cases the longitudinal white line is broken into a series of spots, formed by the mid-anterior and central parts of the upper arm-plates. When pigment is formed very abundantly the white stripe, both dorsally and ventrally, is very narrow and the general coloration is uniform deep purple. Many specimens, however, are not at all deep purple, but are very light colored, or are brownish, reddish, or greenish. This diversity of color is very easily explained. Light-colored specimens are simply those which have developed little pigment. Green specimens are those in which the purple pigment is masked by a green coloring-matter acquired after the formation of the purple; whether this green matter is another pigment or consists of symbiotic algæ I am not prepared to state, as no study of the point was made on living animals. Brown and red shades are produced by the masking of purple by other substances. In all cases, however, purple pigment is the primitive coloring-matter.

The coloration of *Ophiothrix œrstedii* begins, like that of *angulata*, by the development of pigment in the upper part of the side arm-plates. But the pigment spreads laterally and not longitudinally and the whole development of the color-pattern is thus entirely different from that of *angulata* and there is never, and naturally there can not be, an uninterrupted white stripe either above or below. The first bit of pigment to appear may generally be seen in the third or fourth arm-segment in the upper part of the adoral (proximal) portion of the side arm-plate. This rapidly spreads across to meet its fellow from the opposite side and also descends along the side arm-plate ventrally, where it also ultimately meets its fellow from the opposite side and thus completes a narrow purple ring around the arm. Meanwhile another patch of pigment arises just above the upper arm-spine on the side arm-plate and spreads on to the upper arm-plate and down along both sides of the spine-bearing ridge of the side arm-plate. The later behavior of this pigmented area varies more or less in different specimens. In what may perhaps be considered typical *œrstedii*, the two margins (adoral and aboral) of the area accumulate all the pigment and the nearly or quite unpigmented area between them separates them sharply from each other. In such specimens the upper arm-plate is crossed by two heavily pigmented lines, one of which passes down the side arm-plate adoral and the other aboral to the spine-bearing ridge. These lines extend across the under arm-plates and thus form rings encircling the arms. Each arm-segment, in an individual colored like this, has therefore three pigmented rings, one near its aboral margin, distal to the spines, one near its middle just proximal to the spines, and the third at its adoral margin. In many specimens, however, the ring either just distal or just proximal to the arm-spines is indistinct or lacking and the one which is present is very broad on both the upper and under arm-plates. The same obscuring of purple pigment by green and other coloring matters, referred to under *angulata*, occurs in this species also.

It is obvious from the above account that the color pattern of neither of these species of *Ophiothrix* can be derived from that of the other very easily, since one is due to a longitudinal and the other to a transverse distribution of the pigment. While it is true that some specimens of *angulata* have the dorsal white stripe so much broken up, and so much white present elsewhere on each segment, that they are easily confused with *œrstedii*, such specimens do not show definite transverse pigmented rings. Possibly further rearrangement of their pigment might give rise to rings and we could thus say the transverse pattern was evolved from the longitudinal. Evidence, however, that such a development actually occurred is wanting, for *œrstedii* certainly goes through no stage of development wherein it shows any longitudinal distribution of the pigment.

PODIA.

As is well known, the podia of adult *Ophiothrix* are remarkable for their highly papillose surface. It is a matter of some interest, therefore, to note that the podia of the 3 or 4 terminal segments of each arm are perfectly smooth, those of the next 5 or 6 older ones are rough with small papillæ, while it is not until some 9 or 10 segments are developed that the characteristic papillose podia are present.

DISK-COVERING.

One of the most interesting facts in the development of *Ophiothrix* was discovered in the study of the disk-covering. Although no specimens seen were small enough to show simply the primary plates, the specimen only 2 mm. across the disk shows certain primary plates, among which the central one is conspicuous (pl. 3, fig. 18). In a specimen 3.5 mm. across the disk (pl. 3, fig. 19), it will be noticed that the central plate is not only relatively but *actually* smaller than when younger. Evidently a remarkable process of resorption is going on. The extent of this resorption is well shown by the following table:

Diameter of disk in millimeters.	Diameter of central plate.	Length of radial shields.	Ratio of radial shields to central plate.
2	0.60	0.55	0.91
3	.50	1.00	2.00
3.5	.36	1.20	3.33
5	.22	1.85	8.40

Besides the change in size, another change is going on in this plate, during the growth of the disk that is no less remarkable. In the youngest specimen examined the plate is a smooth, thin sheet of lime, entirely unperforated except for a number of minute holes around the margin. As resorption goes on these holes encroach more and more on the plate, not by increase of size so much as by increase of number. When the plate is only 0.25 mm. across, the unperforated area has practically disappeared, and after that stage is reached it is no longer practicable to distinguish the plate from the numerous other small plates at the center of the disk. It can not be said whether the plate is resorbed entirely or whether a minute portion persists. It will be noticed that this plate is utterly unlike anything hitherto reported among echinoderms. Instead of beginning as a triradiate spicule, and steadily increasing in size by dichotomous branching and fusing of the branches, as an echinoderm plate typically does, this plate starts as a large, homogeneous disk which by a process of resorption gradually becomes like its associated plates in both size and structure. In the present state of our knowledge it is futile to speculate on the significance or homologies of this peculiar plate.

Very early in the formation of the disk-covering, but not while the primary plates (excepting indeed the central) are still conspicuous, there begin to arise (in association with the plates) vertical rods trifurcate at their free end. These trifurcate spinelets are usually found one to a plate, but on the radial shields there may be a number, arranged in series of 3 or 4, parallel to the radial margin of each plate. At first there are no spinelets associated with the central plate, but when the disk is about 4 mm. across there may be as many as 5 spinelets upon it. During youth the spinelets are all of approximately uniform height and terminate in 3 sharp teeth of moderate length; but as maturity is reached some of these spinelets grow out into spines, not unlike the arm-spines in length and appearance. They are two or three times as long as the spinelets. The number and distribution of these disk-spines is very diversified and the appearance of the disk is greatly modified by their appearance. As a rule, it may be assumed that the presence of these spines in abundance is indicative of age and their absence is indicative of youth, but the rule is by no means unvarying. The number of spines and spinelets on the radial shields is another variable factor in the appearance of the species. When the radial shields are small (*i. e.*, in the young individuals) a few spinelets (3 to 6) will give them the appearance of being well covered, but as they increase in size, if the number of spinelets does not increase they soon have the appearance of being quite bare. In adults the shields may have very few spinelets, possibly none, and no spines, but it is just as common to find each shield with 10 or 12 spinelets and 3 or 4 spines. The bareness of the radial shields is therefore not a reliable specific character in *angulata*.

MOUTH-PARTS.

The mouth-parts of *Ophiothrix* are essentially different from those of *Ophiactis* and *Amphipholis*, since there are no oral papillæ, but a group of dental papillæ are borne on the lower (ventral) end of the torus, below the teeth. None of my specimens are young enough to show a stage of development in which teeth are present but no dental papillæ, though there can be no possible doubt of the existence of such a stage. In the youngest specimen I have examined, the torus is already expanded at its lower end (pl. 3, fig. 15) and bears a group of 6 or 7 dental papillæ, above which are 4 teeth (pl. 3, fig. 17). Resorption back of the teeth goes on as in the genera already described, but in *Ophiothrix* the foramina thus formed are often bisected by a more or less complete bar at right angles to the long axis of the foramen (pl. 3, fig. 16). This indicates that the fully developed teeth have two slightly separated roots and shows greater specialization than in *Ophiactis* and *Amphipholis*. The number of teeth in *angulata* is 4 or 5, while the number of dental papillæ increases throughout growth, and a large specimen may have as many as 17 (in the big East Indian *Ophiothrix longipeda* there may be more than 30). The first dental papillæ arise in a pair, side by side, below the lowest tooth; beneath them the growing torus expands a little and a new papilla forms near the margin on each side; these two are thus a little separated from each other. A third pair arises below them at the sides of the still expanding torus and the space thus left near the center of the expanded portion of the torus is filled by the formation of a seventh papilla. Subsequently, although the torus may increase much in length, it does not expand greatly and the dental papillæ are thus arranged in two slightly curved marginal rows (one on each side) and an irregular median series. In the adult, resorption has formed foramina back of the upper dental papillæ just as it has back of the teeth (pl. 3, fig. 16). It is perfectly clear, therefore, from all these facts, that the dental papillæ are strictly homologous with the teeth and are not in any way to be homologized with the oral papillæ of other genera. As already stated, there are no oral papillæ in *Ophiothrix*, the oral tentacles being unprotected and conspicuously exposed on the hollowed sides of the jaw.

ARM-BONES OR VERTEBRÆ.

The vertebræ of *Ophiothrix* are as different from those of *Ophiactis* and *Amphipholis* as are the jaws, yet their development is so similar as to call for no especial comment. In the young vertebra of *angulata* (pl. 2, fig. 7), besides the excessive length, we note the very low proximal (adoral) and the very high distal (aboral) ends, the slight development of the alæ, the well-developed aboral and median hypapophyses, the conspicuous protapophysis, and a pair of hollowed projections above the zygosphene, which we may call the *zygocœles*. In the later development of the vertebra the length only increases about one-half, while the height anteriorly increases 3.5 times, and posteriorly more than 6 times. The adult vertebra is about as high as it is long, while the width of the alæ exceeds the total length. Seen from above (pl. 2, fig. 9), the most striking feature is the very narrow protapophysis, the immense zygantrum, the very small and insignificant epanapophysis, the reduced epapophyses (particularly the median one), the broad parapophyses and hypapophyses. Seen from the side (pl. 2, fig. 8) the great height of the protapophysis, the bulging zygocœles, the long zygosphene, and the prominent hypapophyses, all attract attention. Looking at the vertebra from below (pl. 2, fig. 11), the breadth of the taphrus and zygosphene are the most notable features. Looking at the aboral end (pl. 2, fig. 10) we are again struck by the high, narrow protapophysis, the conspicuous zygosphene and the zygocœles, while the broad alæ furnish a fitting background. At the adoral end (pl. 2, fig. 12) the reduced area of the alæ caused by the huge zygantrum, the small zygotreme, within which is a small knob that may be called the *zygophore*, and the large adoral zygapophyses are the principal features. We may then say that the adult vertebra is short and high, as in *Ophiactis*, but, in contrast to that genus, the zygantrum is large, the protapophysis is narrow, high, and projecting, the epanapophysis is insignificant, and the adoral hypapophysis is large.

The joint formed by two vertebræ is more complicated than that in *Ophiactis*. The protapophysis of each vertebra fits into the zygantrum of the next distal segment and a compact mass of muscles, attached at their outer ends to the sides of the cavity, hold the protapophysis in position; the inner ends of these muscles are attached on either side of the protapophysis. The zygocœles serve as sockets for the zygapophyses, while the epanapophysis of the distal vertebra rests lightly on them, the zygosphene meanwhile fitting into the zygotreme and resting against the zygophore. The aboral hypapophyses fit outside and beneath the adoral pair of the distal segment. This articulation is, therefore, considerably more complex than the one described on p. 102 and yet it may permit just as much freedom of movement. The lateral flexures of the arm could certainly be made more vigorously and a curved position held more rigidly than in forms with less-developed zygantra and protapophyses. Possibly the reduced epanapophysis permits a greater freedom in vertical movements, but it may be that the larger adoral hypapophyses counteract this tendency.

Although the development of the vertebra is along the same lines as in *Ophiactis*, there is a constantly increasing difference between that genus and *Ophiothrix*. After the two halves of the vertebra have come to lie close beside each other, the progress of development in the two genera is quite unlike. There is at no time an "*Ophiactis* stage" in the development of the *Ophiothrix* vertebra. No other fact noted has so strongly impressed me with the radical difference between *Ophiothrix* and the other two genera studied.

ARM-PLATES.

In the development of the arm-plates, *Ophiothrix* shows a striking difference from *Ophiactis* and *Amphipholis*. As in those genera, the side arm-plates are the first to appear and are almost immediately followed by the under arm-plate, but in *Ophiothrix* the growth of the under arm-plate is so rapid and vigorous that the side arm-plates never meet ventrally, but are from the first kept apart by the under arm-plates. The latter are in contact with each other throughout the entire length of the arm. The upper arm-plates, on the contrary, are very small near the tip of the arm and it is not until 15 or 20 segments have been formed that they are sufficiently large to separate wholly the side arm-plates. Even at the base of the arm, the basal (adoral) end of the upper arm-plates is so narrow that the side arm-plates of any one segment are not widely separated from each other as they are ventrally. The spine-bearing ridge of the side arm-plates is very well developed, so that the arms appear to be constricted between them, and this appearance is accentuated by the fact that the broader, distal end of each upper arm-plate is somewhat swollen. The side arm-plates, like the vertebræ, are relatively very much longer distally than near the base of the arm.

ARM-SPINES AND TENTACLE-SCALES.

In *Ophiothrix*, as in *Ophiactis*, the lowest arm-spine is the first to appear. It is, on the first segment, a simple, smooth, straight rod with an enlarged base (pl. 3, fig. 1), but by the time a second segment has formed it begins to curve at the tip and becomes broader and somewhat flattened (pl. 3, fig. 2). On the third segment it is distinctly curved at the tip and a tooth is evident on the concave side (pl. 3, fig. 3). On the fourth segment this tooth has become conspicuous and another one has arisen below it (*i. e.*, nearer the base of the spine), while the spine-base is notably large and projecting (pl. 3, fig. 4). On the sixth segment these various features are all accentuated (pl. 3, fig. 5) and on succeeding segments the spine may be said to show its characteristic form (pl. 3, fig. 6). As the animal matures, these lowest arm-spines begin to undergo a sort of retrograde change which well illustrates Hyatt's principle of senescence. Thus if the lowest arm-spine on the twentieth or thirtieth segment from the mouth be examined, on an adult arm, it will be found essentially like that on the twentieth segment from the tip. But if one from somewhere about the twelfth basal segment be looked at (pl. 3, fig. 7), it will be found to be obviously different from any of the lowest arm-spines of the middle portion of the arm; except that it is immensely stouter, it is more like that of the fourth segment from the arm-tip. It has one notable peculiarity in that foramina are beginning to open through it. Examination of the lowest spine of segment nine, counting from the mouth, shows (pl. 3, fig. 8) a still further reduction of teeth, particularly the terminal one, while there is a corresponding development of the foramina. The lowest spines of the segments still nearer the mouth (pl. 3, figs. 9 and 10), show these tendencies carried still further. Indeed, except for their smaller size, these basal lowest spines are not essentially different from the ordinary spines of the arm.

The development of the other arm-spines offers no such interesting features as those shown by the lowest. The next to the lowest is the second one to appear and is generally present on the second segment. The others appear in regularly vertical sequence dorsally. The third spine is to be found on the fourth segment from the tip and the fourth on the ninth. It is not until about 40 segments are developed that a fifth spine appears, while the arm has no less than 60 joints when the sixth spine is to be seen. A seventh spine will arise if about 75 joints are formed, and an eighth if the number is about 90. I have not found more than 8 spines in any specimen, and think that 6 or 7 is the characteristic number for the species.

The tentacle-scales are reduced to very narrow, sharp spinelets, which are quite lacking at the tip of the arm. The first indication of them is on the sixth or seventh segment, where a minute one is to be found on each side, connected with the lowest part of the side arm-plate. They never reach a large size, but even on the basal segments give the impression of being functionless remnants. There is no indication whatever of homology with an arm-spine.

SUMMARY OF GROWTH CHANGES IN OPHIOTHRIX ANGULATA.

1. Although the coloration is distinctive, it is acquired very early without passing through any recognizable stages. It is due to the deposit of purple pigment in the arm-segments *longitudinally* and the usually complete failure of any pigment to cross the median line either dorsally or ventrally. When the color is other than some shade of purple, it is due to the masking of the purple pigment by a secondarily added color.
2. The first-formed podia are smooth, as is usual in ophiurans, and the papillose condition characteristic of the genus *Ophiothrix* is only acquired after about 10 segments are complete.
3. The central plate of the youngest specimens examined is a disproportionately large, homogeneous, thin disk with a few minute perforations around the margin. During the growth of the disk this plate is gradually resorbed, becoming not only much smaller (actually as well as relatively) but more and more reticulate from the increasingly numerous foramina.
4. The disk-plates carry trifid spinelets, which not only become more numerous with growth of the disk, but have a tendency to elongate into slender spines. The number of these disk-spines is very much subject to individual diversity.
5. There are no oral papillæ in *Ophiothrix*, but after 4 or 5 teeth are formed, dental papillæ (homologous with the teeth, but much

smaller) arise in double and then in triple series ventral to the teeth. The torus expands correspondingly, and in maturity the lower dental papillæ may be in irregular quadruple or quintuple series, though the *lowest* series are triple or only double, like the highest. Resorption of the torus occurs back of the largest dental papillæ just as it does back of the teeth.

6. The vertebræ are remarkable for the high, narrow protapophysis anteriorly and the large zygantrum and very small epanapophysis posteriorly. The chief articulation between two vertebræ is formed by the protapophysis of one and the zygantrum of its distal fellow. Other peculiarities of the mature vertebræ are a pair of zygocœles above the zygosphene and a small, round knob, the zygophore, in the zygotreme. The zygapophyses and adoral hypapophyses are well developed.
7. The side arm-plates are separated throughout the whole length of the arm, even in the first segment, by the under arm-plates, so that ventrally the two side arm-plates of any given segment are never in contact. Dorsally those of the first 15 or 20 segments do meet each other in the mid-dorsal line, but thereafter the upper arm-plates keep them apart. The spine-bearing ridges of the side arm-plates are quite prominent, especially on the older segments.
8. The lowest arm-spine, the first to arise, begins as a straight, slender rod, but rapidly develops into a characteristic 3-toothed hook. At the base of the arm, by a process of senescence, it is gradually transformed into a nearly straight, rough, fenestrated spine like those dorsal to it.
9. There are only 4 (distally fewer) arm-spines on the first 40 segments and the full number for the species (6 or 7) does not appear until 60 or 75 segments are complete.
10. The tentacle-scales are reduced to mere spinules associated with the basal part of the side arm-plate. They do not appear until after 6 or 7 segments are formed and they never seem to be of any functional importance.

SUMMARY AND CONCLUSIONS.

Although references to changes in appearance, or in details of structure, during the transition from young to adult brittle-star are to be found in many of the writings of Lyman, Lütken, Verrill, Grieg, Koehler, de Loriol, and other less well-known workers, no attention to the sequence or significance of these changes has been paid, up to the present time, save by Ludwig and Mortensen. And although it has been a generally accepted and well-known fact that the terminal segments of the adult ophiuran arm are the youngest, no use has been made of the important corollary that these segments reveal the developmental stages through which the arm has passed. The present report has endeavored to make full use of this corollary in studying the development of *Ophiactis*, *Amphipholis*, and *Ophiothrix*. Its great value lies, of course, in the fact that even where only a single specimen of a species is available, and that specimen adult, if a single arm is complete to its terminal plate the development of that species throughout its post-larval growth, so far as the arms are concerned, can be read almost as plainly and satisfactorily as if a large series of young specimens were available. Obviously this is not true of the disk. I would emphasize, therefore, as the first conclusion of these studies, this truth of the very great importance of localized stages in the distal part of the ophiuran arm. The other conclusions which follow are taken directly from Ludwig's very valuable paper "Jugendformen von Ophiuren" (1899), supplemented by Mortensen's observations on *Asteronyx* (1912) and by my own work. The following list shows the species of brittle-stars, the growth-changes of which have been more or less fully studied:

Ophiactis asperula (Philippi).
kröyeri Lütken.
savignyi (Müller & Troschel).
Amphipholis patagonica Ljungman.
squamata (Delle Chiaje).
Amphiura magellanica Ljungman.
Ophiacantha vivipara Ljungman.
Ophiothrix angulata (Say).
Ophiomyxa vivipara Studer.
Asteronyx loveni Müller & Troschel.

This list strikingly demonstrates the paucity of facts on which to base a classification of brittle-stars. We have a fair knowledge concerning the growth-changes of 10 of the species (out of approximately 1,100!), but these represent only 7 genera and perhaps 5 families. It may be added that a considerable number of facts are recorded concerning changes during development from young to adult of several euryalids (*Euryale*, *Gorgonocephalus*), but in no one species has any series of changes been followed.

The following conclusions, a summary of our knowledge to date, will serve as a basis for further work and may prove useful in future attempts to properly locate doubtful genera and difficult species. Continued study of growth-changes will ultimately lead to a natural classification of ophiurans.

1. The terminal segments of the arm are the youngest; passing from the terminal plate towards the disk, each succeeding segment is older. The segments in their individual development pass through the same stages that the arm has passed through in its development and therefore the terminal portion of the arm is made up of *localized stages*, to use Jackson's (1899) expressive term, which reveal the developmental or ontogenetic stages of the arm as a whole. The study of the terminal portion of the arm in great detail is necessary therefore to the proper understanding of the relationships of any brittle-star.
2. The original disk-covering in ophiurans consists of a central and 5 radial plates; interradial plates are a somewhat later addition; the radial shields still later; further development of both radial and interradial plates between the primary plates is a more advanced stage; the development of spinules and spines in connection with the disk-plates is further evidence of specialization; and the resorption of the disk-plates or their concealment by thick skin or by a coat of granules must be regarded as still more advanced stages in the development of disk-coverings.
3. The *radial shields* are originally small and comparable to any of the other secondary disk-plates. Their development to a large size is evidence of specialization. The larger the radial shields in proportion to the other disk-plates, the more specialized is the disk-covering, other factors (such as granule formation) being equal.

4. The *oral shields* lie originally on the dorsal surface and only by subsequent developmental changes come to lie on the ventral side. The apparent exception shown by *Asteronyx* seems to me an undoubtedly secondary condition; this is indicated by their late and unequal appearance.
5. The *adoral plates* develop from the second pair of adambulacral (= side arm) plates. A large size *relatively*, as compared with the oral shields, is a primitive condition, but a large size *actually*, so that they are equal to or exceed the basal side arm-plates, is a secondary and specialized condition. On the other hand, their great reduction or entire absence is also a very specialized condition.[1]
6. The jaws (*oral plates*) arise, in large part at least, from the first pair of adambulacral plates. Obviously the more clearly they show their origin the more primitive is their character, while the more completely they are fused and overshadowed by either the torus or the adoral plates, the more specialized is their condition.
7. The *torus* arises, at the tip of the jaw, as a small plate not much higher than wide. A low, wide torus, therefore, indicates a primitive condition, while a high, narrow one or a high one expanded at the lower end indicates specialization.
8. The *teeth* arise in association with the torus, but not as a part of it. The uppermost tooth of the adult was the first one developed, the later teeth appearing below it successively. Obviously, a small number of teeth is a primitive condition, and the same is true where the lower teeth are evidently smaller than the uppermost. Young teeth are sharply pointed; therefore pointed teeth are evidently more primitive than those which are rounded or truncate. A large number of truncate teeth of equal size shows a decidedly specialized condition.
9. The *dental papillæ*, where they occur, are homologous with the teeth, but are a modified form of the latter. Their occurrence is therefore undoubtedly a mark of specialization, the degree of which may be indicated by their number and arrangement.
10. The *oral papillæ* arise in connection with the first and second adambulacral plates (*i. e.*, the orals and the adorals) and appear to be homologous with tentacle-scales. There is as yet no evidence to determine whether their entire absence is a primitive or highly specialized condition. Possibly it may be either. There is evidence to show that at least a relatively primitive condition is indicated when there is one papilla in connection with each oral and each adoral plate. There can be scarcely any doubt that the presence of more than one papilla in association with each of these plates indicates specialization, the degree of which is suggested by the number of such papillæ. The primitive form of oral-papillæ appears to be scale-like, flat with rounded tip; pointed and spine-like papillæ are specialized forms.
11. The *genital-slits* or bursal openings appear very early, one on each side of the base of the arm. The evidence is still lacking as to whether

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[1] In Mortensen's (1912) very valuable and interesting account of growth-changes in *Asteronyx*, he speaks of the "seitenmundschilder" being resorbed and later as being greatly reduced. But examination of his admirable figures shows there has been no resorption; the fact is that having reached a relatively large size quickly, their later growth has been slower than that of the rest of the disk; thus in his specimen 1.9 mm. across the disk, the adoral plates are each 0.55 mm. long, in the specimen 3.5 mm. across the disk, they are 0.60 mm. long, and in the specimen 25 mm. across they are 3 mm. long. Surely there is no resorption here.

their absence is a primitive or specialized condition. Perhaps it may be either. In any case, the fusion of the margins of the slit so as to form *two* openings on each side of the arm-base is undoubtedly a highly specialized condition.

12. The arm-bones or *vertebræ* arise by the fusion of two skeletal pieces lying side by side above the radial water-tube. They are at first relatively elongated and low, with the two component halves distinguishable and the articulation resembling a simple ball-and-socket joint. Specialization is indicated by loss of indication of the bilateral origin, by greatly increased height, and hence a relatively short longitudinal axis, a greatly increased number of elevations, projections, and ridges for the attachment of muscles, and an articulation complicated by development of zygapophyses, protapophysis, and epanapophysis. Owing to changes from compression and other causes the vertebræ of the basal arm-segments do not reveal the typical form for the species. At about 8 or 10 segments from the mouth the vertebræ of an adult ophiuran with 40 or more arm-joints are fairly typical, but after a dozen, or even fewer (in less mature individuals) segments further out, youthful characters begin to show and the vertebræ become less and less typical as we approach the terminal segments of the arm.

13. The *terminal* plate of the arm is the first formed. It is originally a flat or concave plate on the upper side of the tip of the water-tube. It grows down and around the tube and by ventral union becomes a cylinder. The distal margin of this cylinder may bear one to several teeth or spinelets. It may become enlarged proximally and even conspicuously cup-shaped, but such changes indicate specialization of some kind. The most primitive condition is undoubtedly a simple plate or a half-formed cylinder; a completed cylinder shows at least some specialization.

14. The *side arm-plates* are the first plates of an arm-segment to appear. They grow rapidly and soon meet each other in the mid-dorsal and mid-ventral lines, where they are in close contact, although they do not fuse. They inclose the rudiments of the vertebræ, which do not arise until they are well begun. In later development they may be more or less separated from each other ventrally by the development of the under arm-plates and dorsally by the upper arm-plates. At first they are low and longitudinally elongated, the whole segment being long and flat. In their usual development the height increases much more rapidly than length and they consequently tend to become short and high. The distal margin is somewhat thickened and bears one or more arm-spines. It may become notably enlarged and a conspicuous spine-bearing ridge may be developed, which in the course of growth comes to lie in the median vertical region of the plate. The distal margin of the lower end of each side arm-plate may be more or less deeply grooved for the passage of the podium (tube-foot) and usually a tentacle-scale arises at the same point. Evidently then, long, low side arm-plates, meeting above and below and with no spine-bearing ridge or tentacle-groove, show a primitive condition of the arm-segment, while short, high side arm-plates separated above and below, with a conspicuous spine-bearing ridge and a noticeable tentacle-groove, indicate considerable specialization. All conditions between these two extremes may be found.

15. The ***under arm-plate*** arises very soon after the formation of the side arm-plates and may even precede the formation of the vertebral rudiments. It is at first small and lies on the oral side of the arm, in the median line just distal to the side arm-plates. It may grow backward (adorally) sufficiently to completely separate the side arm-plates and it then comes in contact with its fellow on the succeeding segment. In such cases the under arm-plates are more or less clearly in contact throughout the entire length of the arm. There is no doubt that small, widely separated under arm-plates are the simple, primitive arrangement and large under arm-plates in contact with each other indicate specialization. The absence of under arm-plates or their occurrence in irregular series and of diverse sizes are certainly indicative of specialized conditions.

16. The ***upper arm-plates*** are the last of the arm-plates to arise, but they appear usually not long after the under arm-plates and follow a similar line of development. Small, widely separated upper arm-plates are undoubtedly a primitive condition, while large upper arm-plates, in contact with each other and separating the side arm-plates, are certainly specialized. On the other hand, absence of upper arm-plates, if associated with large side arm-plates broadly in contact in the mid-dorsal line, shows a primitive type of structure, while absence of upper arm-plates if associated with widely separated side arm-plates is probably a highly specialized condition.

17. The ***lowest arm-spine*** is the first to appear, as a rule, though the one just above it may accompany or, in very rare cases, slightly precede it. Succeeding spines arise dorsal to the earlier-formed and the number increases slowly with the age of the segment until the number characteristic of the species is attained. This number is commonly to be found on about the tenth segment from the mouth and thence distally for a variable number of segments. On the basal arm-segments the number is always fewer than normal, but whether the influence of the disk has promoted resorption or prevented formation of the full number has not been ascertained.

18. The ***hook-shaped form*** of the young lowest arm-spine is not characteristic of any particular family of brittle-stars, but its persistence and perfecting on the mature segments of the middle arm may be a family or at least a generic character. Young spines arise as straight rods, but soon become hooked or give rise to tooth-like projections. They usually acquire a secondary straightness. Young spines are solid, but may become hollow with increasing size. Hollow spines undoubtedly indicate a specialized condition.

19. The ***tentacle-scales*** may arise as early as the under arm-plate and before the lowest arm-spine, or their appearance may be delayed until after several spines have been developed. Each tentacle-scale is at first flat and rounded and is associated with the ventral, distal margin of the side arm-plate. It may be homologous with an arm-spine, but its position ventral to the lowest arm-spine counts against that view. The evidence is as yet inconclusive whether the absence of tentacle-scales is a primitive condition or not. When two tentacle-scales are present, the second one arises in connection with the under arm-plate a little later than the first one. Its occurrence is almost certainly a specialized condition and it can not be homologized with an arm-spine. A spine-like tentacle-scale is certainly a modified form.

20. The *podia* arise as smooth, paired outgrowths of the radial water-vessel just proximal to the terminal plate. Each one is associated with a side arm-plate. They ordinarily remain smooth throughout life, but in some brittle-stars they are rough, minutely or conspicuously papillose. Such changes are obviously specializations and such podia are often less retractile than usual.
21. The number of arm-segments included in the disk increases with age and such segments are commonly more or less modified by their position.
22. The *number of arms* is determined when the metamorphosis occurs or when the typical ophiuran form is assumed. New arms never arise subsequently, except in schizogonous species, when they develop in connection with the new disk formation. A 5-rayed individual never becomes 6-rayed after its 5 arms are fully formed nor does a 6-rayed individual ever become 5-rayed by autotomy and resorption, as has sometimes been supposed. A 5-rayed or 6-rayed young brittle-star will remain such throughout life unless schizogony occurs.

MUSEUM OF COMPARATIVE ZOÖLOGY,
Cambridge, Mass., March, 1913.

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LIST OF PLATES.

EXPLANATION OF LETTERING.

A = Ala.
AH = Anterior hypapophysis.
E = Epanapophysis.
Ep = Epapophyses.
LAR = Lower alar ridge.
MH = Median hypapophysis.
P = Protapophysis.
Pa = Parapophysis.
PH = Posterior hypapophysis.
T = Taphrus.
UpAR = Upper alar ridge.
Z = Zygosphene.
Zc = Zygocœle.
Zg = Zygapophysis.
Zp = Zygophore.
Zt = Zygotreme.
Zy = Zygantrum.

Plate 1.

Ophiactis savignyi (M. & T.).

Fig. 1. A young individual, showing bases of 3 arms, and 3 very youthful arms, of which the median is evidently the youngest. ×40.
2. Disk of smallest individual seen, showing primary plates on older portion. ×40.
3. Mouth of a young specimen, showing 2 old jaws and 3 pairs of old oral tentacles, and 4 young jaws. ×90.
4. Side view of a young jaw with 4 teeth and an old jaw with 5; 2 oral tentacles are also shown. ×90.
5. Torus of a young individual. ×90.
6. Fourth segment from tip of arm, showing skeletal parts, as seen from below. ×90.
7. A very young arm, with only one segment; seen from below. ×90.
8. Vertebra of fifty-fifth segment from mouth in an arm with 70 joints; seen from above. ×40.
9. Vertebra of fiftieth segment of same arm; from above. ×40.
10. Vertebra of fortieth segment of same arm; from above. ×40.
11. Vertebra of thirtieth segment of same arm; from above. ×40.
12. Vertebra of tenth segment of arm with only 18 joints; from above. ×90.
13. Vertebra of twentieth segment of arm with 70 joints; from above. ×40.
14. Vertebra of tenth segment of same arm; from above. ×40.
15. Same vertebra as fig. 14; seen from aboral end. ×40.
16. Same vertebra; seen from below. ×40.
17. Same vertebra; seen from adoral end. ×40.
18. Same vertebra; seen from side. ×40.
19. Vertebra of second segment of arm with 70 joints; seen from above. ×40.

Plate 2.

Figs. 1 to 6.—*Amphipholis squamata* (Delle Chiaje).

Fig. 1. One of the second pair of adambulacral plates, with its adjoining oral tentacle, from a specimen 0.65 mm. across the disk; to show posterior (aboral) spinous projection. ×375.
2. One of the oral plates ("jaw") and its adjoining adoral plate, from a specimen 0.80 mm. across disk; to show origin of first 2 oral papillæ, in connection with them, but from separate centers of calcification. ×375.
3. A jaw, and adjoining parts, from a specimen 1 mm. across disk; to show *Amphiura*-stage of development. ×90.
4. A jaw, and adjoining parts, from a specimen 1.35 mm. across disk; to show the *Amphipholis* character attained.
5. Side view of torus and first tooth, from a specimen 0.65 mm. across disk. ×375.
6. Side view of torus and first tooth, also from a specimen 0.65 mm. across disk but somewhat older. ×375.

Figs. 7 to 12.—*Ophiothrix angulata* (Say).

Fig. 7. Vertebra of sixth segment from tip of arm; from the side. ×90.
8. Typical vertebra; from the side. ×40.
9. Same vertebra; from above. ×40.
10. Same vertebra; showing aboral surface. ×40.
11. Same vertebra; from below. ×40.
12. Same vertebra; showing adoral surface. ×40.

PLATE 3.

FIGS. 1 to 10.—*Lowest arm spine of Ophiothrix angulata (Say).* ×100.

FIG. 1. From first (next to terminal plate) segment.
2. From second segment.
3. From third segment.
4. From fourth segment.
5. From sixth segment.
6. From middle portion of the arm (any segment).
7. From twelfth segment, counting from mouth.
8. From ninth segment from mouth.
9. From sixth segment from mouth.
10. From third segment from mouth.

These spines are all shown in the same position. Figure 6 shows the appearance of the typical spine characteristic of the genus; figures 1–5 show its development, and figures 7–10 its senescence. Spines, like figures 9 and 10, are very little different from the ordinary arm-spines.

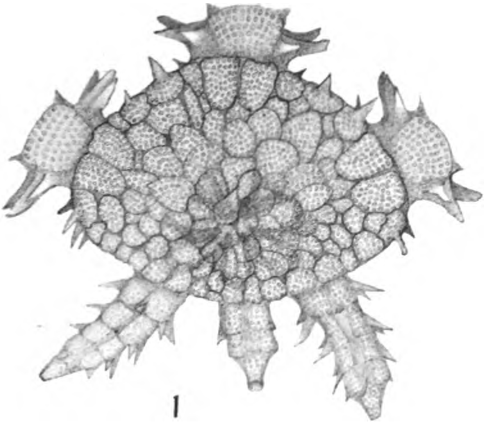
11. Next to the lowest arm-spine of *Amphipholis squamata* (Delle Chiaje) from third segment, counting from tip of arm. ×375.

FIGS. 12 to 14.—Lowest arm spine of *Ophiactis savignyi* (M. & T.). ×375.

FIG. 12. From third segment from tip of arm.
13. From sixth segment.
14. From middle of arm (any segment).

FIGS. 15 to 19.—*Ophiothrix angulata* (*Say*).

FIG. 15. Torus of specimen 2 mm. across disk. ×90.
16. Torus of adult, 5 mm. across disk. ×40.
17. Torus, teeth, and dental-papillæ of young, 2 mm. across disk, seen from the side. ×90.
18. Central plate, radial shields, and part of disk of a specimen 2 mm. across disk. ×40.
19. The same of a specimen 3 mm. across disk. ×40.



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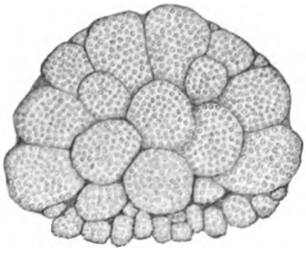

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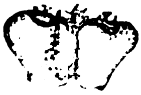

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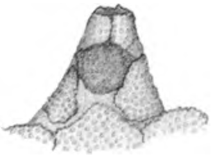

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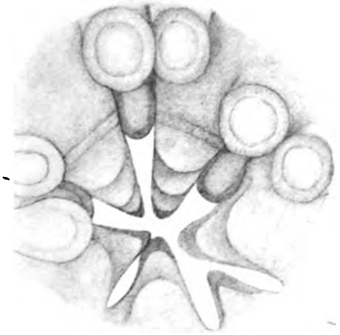

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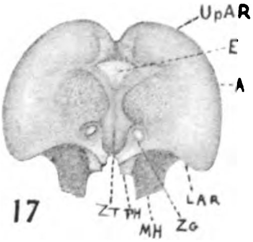

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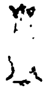

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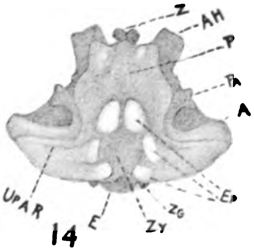

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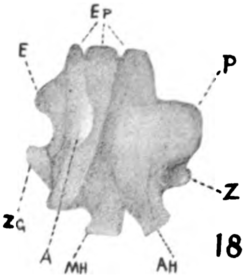

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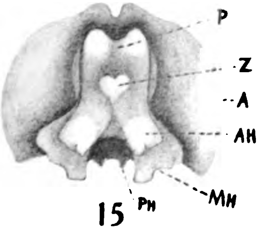

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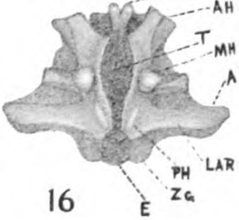

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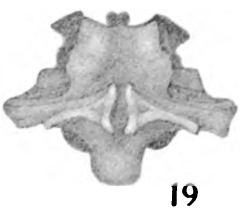

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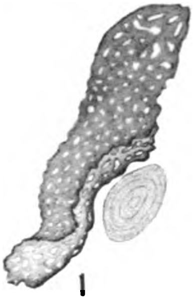

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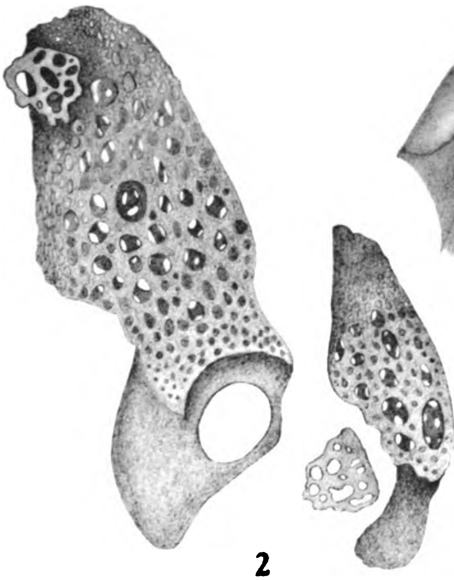

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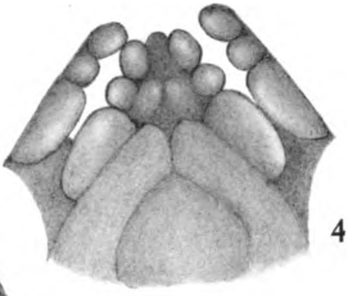

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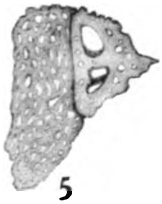

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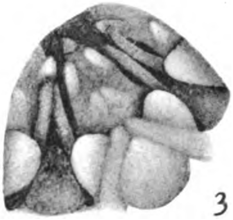

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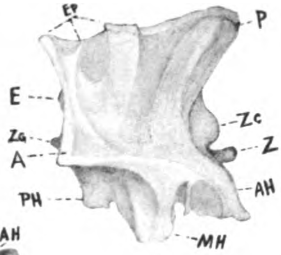

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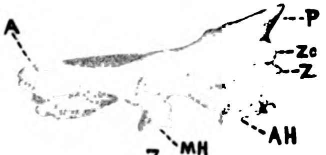

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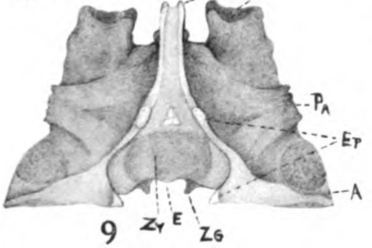

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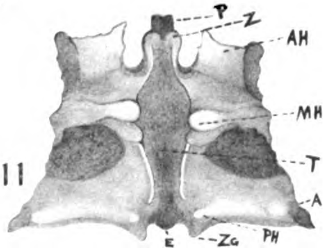

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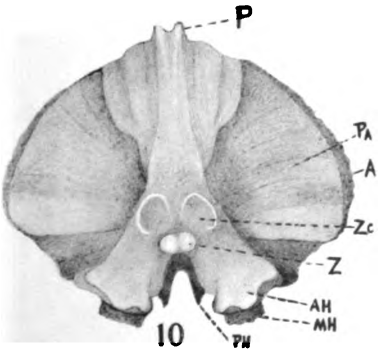

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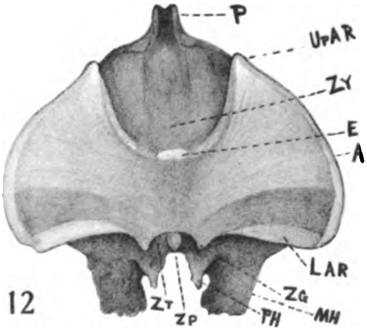

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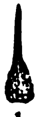
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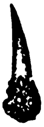
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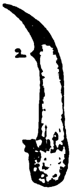

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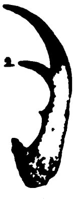

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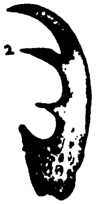

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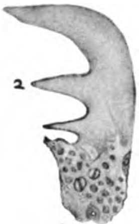

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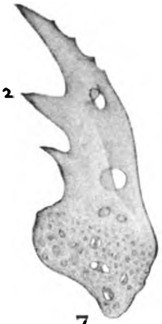

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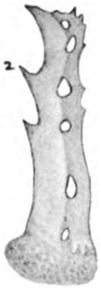

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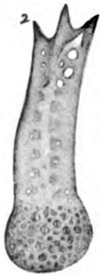

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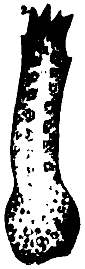

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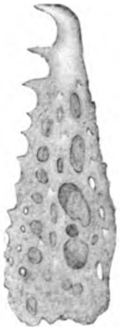
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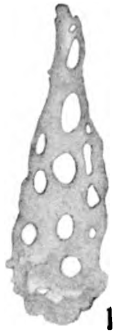
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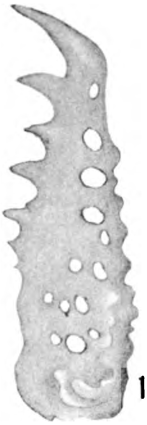
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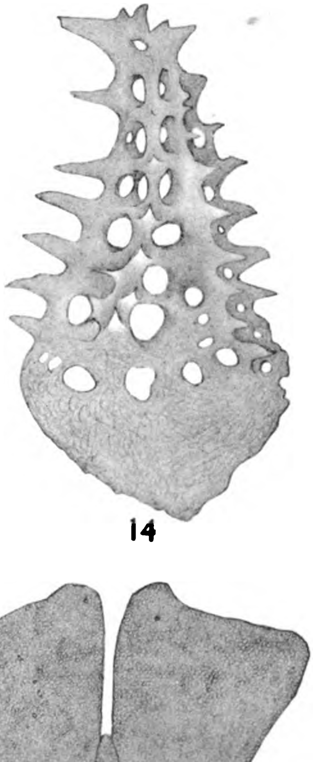
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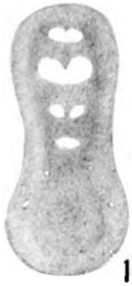
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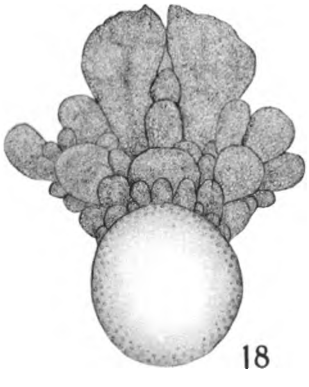
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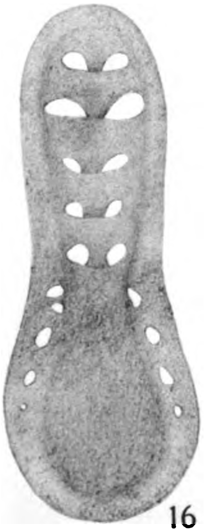
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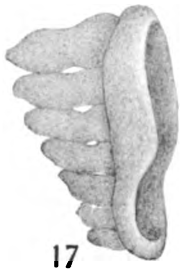
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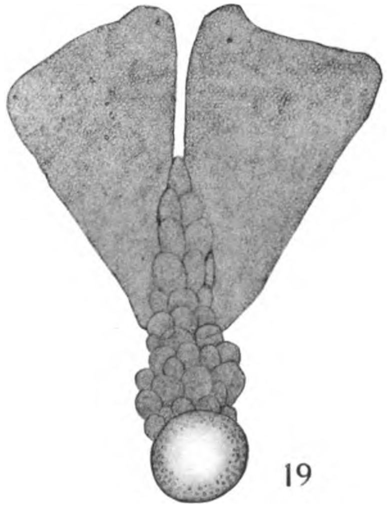
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VIII.

THE EARLY INFLUENCE OF THE SPERMATOZOAN UPON THE CHARACTERS OF ECHINOID LARVÆ.

BY DAVID H. TENNENT,
Bryn Mawr College.

Eleven text-figures.

THE EARLY INFLUENCE OF THE SPERMATOZOAN UPON THE CHARACTERS OF ECHINOID LARVÆ.

BY DAVID H. TENNENT.

The question of the influence of the spermatozoan upon the character of the larva has been the basis of a vast amount of discussion among students of embryology. From the time of the early disagreement between the animalculists and the ovists it has proved a fruitful subject for speculation.

In more recent times, since the evolution of the school of developmental mechanics, various attempts have been made at an exact determination of the rôle played by the spermatozoan or by some of its parts. It is conceded that the characters resident in the male parent are transmitted to the offspring by the spermatozoan, either by the chromatin or by the cytoplasm, or possibly by both. But there is no general agreement as to the time in the individual history when "paternal" characters may become evident. There is a fairly general opinion that the egg conditions development, that it is responsible for at least the early developmental processes, and that the sperm, although instigating development, places no paternal stamp on the offspring until relatively late.

Boveri's (1895) classical experiment, criticized adversely by various investigators, but confirmed by Herbst (1907), in which enucleated egg fragments of one species of sea-urchin, fertilized by sperm of another species, gave rise to larvæ which were paternal with respect to the skeleton, showed that in the absence of female nuclear material the sperm nuclear material might assume the direction of some phases of development by the time of the pluteus stage, but there has been little evidence, one way or the other, regarding younger stages. Here the evidence has been negative in so many instances that a positive generalization is often heard to the effect that in development the influence of the spermatozoan is not shown in the character of early embryos.

The discussion between Boveri and Driesch on this subject, in 1903–04, resolved itself finally into a simple difference of opinion in regard to the earlier or later time at which certain characters made their appearance in Echinoid hybrids. Boveri (1895, 1903, 1904) presented evidence on the form of the larvæ, the skeleton, the number of chromatophores, the pigment content of the chromatophores, the arrangement of the chromatophores, the number of primary mesenchyme cells, and, under certain circumstances, the size of the larvæ. Driesch (1903) considered the same points, but by the evidence neither was able to change essentially the belief of the other.

About five years ago the controversy was given a somewhat different form as a result of the researches of Loeb (1908) on heterogeneous hybridization. In this work an echinoid egg, *Strongylocentrotus*, was fertilized by molluscan sperm, *Chlorostoma*, and the resulting plutei were taken as evidence that the inheritance was purely maternal, *i. e.*, from the egg alone. The reason for this was shown by Kupelwieser (1909), who worked out the cytology of the cross *Echinus microtuberculatus* female by *Mytilus galloprovincialis* male and was able to show that true fertilization did not take place, the male nucleus never fusing with the female nucleus, but instead lying at one side of the mitotic figure during division, the larvæ resulting from the cross being, in effect, parthenogenetic.

Herbst (1906) obtained a closely similar result by a combination of artificial parthenogenesis and fertilization, in which the larvæ were held to the maternal type. During segmentation the paternal nuclear material lags and may fail to take part in the segmentation. Development in such cases may be in reality maternal, but it is parthenogenetic.

Results again resembling these were obtained by Günther Hertwig (1912), when he inseminated sea-urchin eggs with spermatozoa which had been subjected to radium rays and found that here again the male nucleus failed to take a normal part in the processes of fertilization and cleavage.

We must distinguish between the cases afforded by this work of Loeb, Kupelwieser, and Hertwig, which deals in fact with a type of parthenogenetic development, no fertilization taking place, and cases in which true fertilization occurs, *i. e.*, the union of an egg and sperm nucleus, although here again we must distinguish between cases in which the union is only temporary and those in which it is permanent. From work done by Baltzer (1909, 1910), Herbst (1909, 1912), and Tennent (1912), evidence has been gained which has proved that even though the two nuclei unite, some of the chromosomes may be eliminated later.

In Godlewski's (1906) fertilization of the echinoid egg with crinoid sperm the union of the nuclei seems to have been permanent. In his fertilization of enucleated fragments of sea-urchin eggs with crinoid sperm, the young stages (gastrulæ, four cases) were larvæ which followed the sea-urchin type.

Finally "partial fertilization," discussed by Boveri and Herbst, also represents a true fertilization, although the union between the nuclei may be delayed until after segmentation has begun, and the resulting pluteus may be in part maternal, in part hybrid in nature.

The consideration of these points makes it clear that we have no established evidence of the influence of the sperm on the characters of stages younger than the pluteus and that new observations based on favorable material is desirable. I count myself fortunate in obtaining material of this nature while on the expedition made by the Department of Marine Biology to Montego Bay, Jamaica.

MATERIAL AND METHODS.

One of the abundant reef sea-urchins at Montego Bay is the primitive form *Cidaris tribuloides*. The living egg of *Cidaris* is exceedingly transparent and the union of the male and female nuclei may be followed in it without difficulty; it may be fertilized readily in the laboratory with its own sperm or with the sperm of other sea-urchins abundant in the vicinity, among these being *Toxopneustes* (*Lytechinus*) *variegatus* and *Hipponoë* (*Tripneustes*) *esculenta*. These crosses are easily made. The eggs may be fertilized at once after their removal from the gonad, without artificial aid, although as a check against the possibility of chance fertilization with *Cidaris* sperm they were kept for 2 hours before making the fertilizations. A portion of the eggs was always kept as an unfertilized control.

Many investigators will recognize the convenience and advantage of supplying developing larvæ with an almost complete change of water from time to time. I found it possible to do this by centrifuging the contents of the finger bowls, which I used as aquaria, withdrawing the water from above the larvæ and replacing with fresh water. This method was used only after the third day. This material was centrifuged only long enough to throw the larvæ down to the end of the tubes. The plutei treated in this manner kept in better condition than those not centrifuged and thus not given as complete a change of water. Owing to my limited time no attempt was made to rear the larvæ to metamorphosis. Every possible precaution was taken to avoid chance fertilization or contamination of any nature of the cultures.

Since the completion of the investigation is being delayed by the pressure of teaching it seems best to present at this time the results that were obtained with the living material, leaving the cytological analysis of fixed material for a later time. The outline figures used in illustrating the paper are from camera-lucida sketches made from living larvæ.

INVESTIGATION.

In its normal development *Cidaris* proved of interest, (1) because of its slowness of development when compared with *Hipponoë* or *Toxopneustes;* (2) in the difference in the site of its mesenchyme formation; (3) in the place of the appearance of the larval skeleton.

The anaphase of the first cleavage begins about 50 minutes after insemination. The cleavage is like that of other echinoids. The blastula stage is reached in 16 to 18 hours, depending on the lot of eggs; gastrulation begins in 20 to 23 hours; mesenchyme formation begins in 23 to 26 hours, the mesenchyme cells arising from the inner end of the archenteron; chromatophores appear in about 44 hours; the enterocœle arises as a single pouch in 44 to 50 hours; in 55 hours two enterocœles may be seen, formed by the division of the single vesicle; in 72 to 73 hours the beginning of the formation of the skeleton may be noted. Even at this time, the beginning of the fourth day, the body has not begun to assume the form of an echinopluteus, and it is not until the fifth day that the arms begin to push out. These facts are of interest when compared directly with those regarding the development of *Toxopneustes.*

The processes are here shown in parallel. The hours mentioned indicate hours after fertilization.

Cidaris.

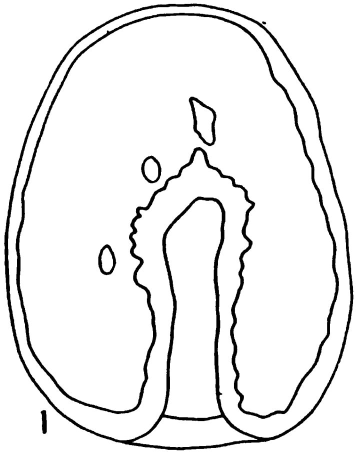
	Hours.
Blastulæ (swimming)	16 to 18
Gastrulæ (beginning)	20 to 23
Mesenchyme	23 to 26
Chromatophores	44
Skeleton (beginning)	72 to 73
Pluteus	120

Toxopneustes.

	Hours.
Blastulæ (swimming)	5.5
Mesenchyme	8
Gastrulæ (beginning)	9
Chromatophores	15 to 16
Skeleton (beginning)	15 to 16
Pluteus	24

The earliest striking difference between the two forms is in the time and place of mesenchyme formation, *Cidaris* in this respect resembling the crinoids more closely than the echinoids.

No mesenchyme is formed until the archenteron has pushed well into the blastocœle (fig. 1). The cells then push out from the end and from just behind the end of the archenteron. During the next few hours the migration of cells from the archenteron continues, while the cells within the blastocœle increase in number by division. A network formed by fine protoplasmic processes, as in other echinoids, is formed (fig. 2).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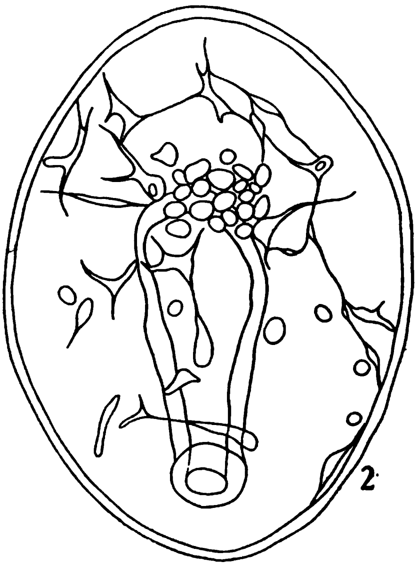
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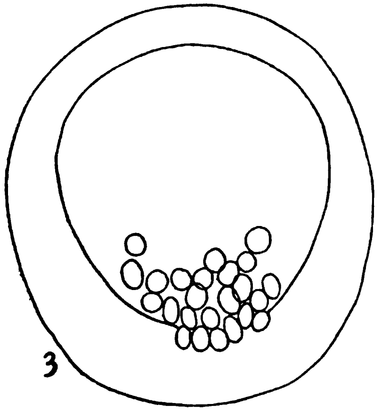
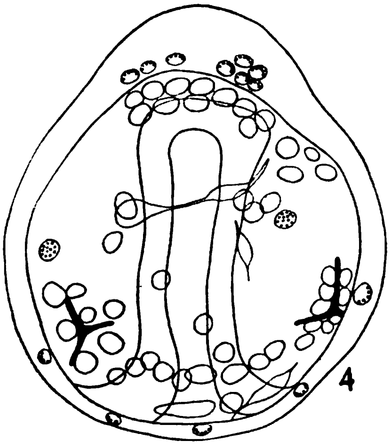
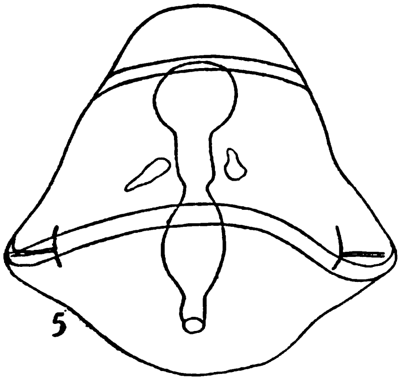
FIG. 1.—Optical section, *Cidaris* gastrula, showing beginning of mesenchyme formation. 23 hours.
FIG. 2.—Optical section, later *Cidaris* gastrula. 41 hours.

In *Toxopneustes*, it will be recalled, the micromere end of the blastula becomes flattened and the primary mesenchyme cells push into the blastocœle before gastrulation begins (fig. 3). This mesenchyme is first scattered throughout the blastocœle, the greater number of the cells remaining, however, at the posterior pole. When the archenteron pushes in they lie as a ring about it, later becoming aggregated in two groups, one at each side of the base of the archenteron; in each group the characteristic skeletal spicules arise. The secondary mesenchyme is given off from the end of the archenteron after this has grown well toward the anterior end of the blastula (fig. 4).







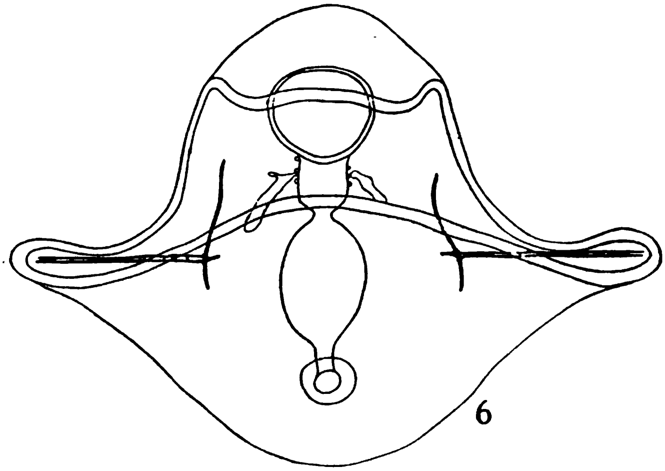


FIG. 3.—Optical section, *Toxopneustes* blastula, showing beginning of primary mesenchyme formation. 8 hours. Drawn from preserved material.
FIG. 4.—Optical section of *Toxopneustes* gastrula, showing formation of secondary mesenchyme and of skeleton. Chromatophores in blastocœle and wall. 41 hours.
FIG. 5.—Young *Cidaris* pluteus, showing character and position of early skeleton.' 73 hours.
FIG. 6.—*Cidaris* pluteus. 6 days.

In the case of *Cidaris*, after the formation of the primary enterocœle and the dorsal water-tube (50 hours), the gastrula begins to elongate and to become broader at the posterior end. When the primary enterocœle separates into right and left enterocœles (the left remaining in connection with the water-tube (55 hours),two ciliated bands make their appearance, the posterior band about one-third the length of the body from the posterior end of the body, the preoral band somewhat less than one-third the length of the body from the anterior end. Between these bands the body now begins to be somewhat concave. In general appearance at this time the larva resembles that of *Asterias* more than it does that of an echinoid. At 66 hours the bands are well developed and the region between the bands has become still more concave.

Between this time and 72 hours a further increase in size and change in form of the body is apparent, the posterior half becoming much broader and the posterior end more rounded, while by reason of this change of form the anal region has been shifted forward. During the period from 60 to 65 hours the œsophagus, stomach, and intestine differentiate from the archenteron. About 72 hours after fertilization the first indication of a larval skeletal system may be noted in the triradiate spicules arising in groups of mesenchyme cells in the broadest part of the body, about the level of the anterior half of the stomach, and just beneath the postoral band of cilia. The laterally directed axis is seen to be double and gives rise to a rod of the latticed type (fig. 5). During the next day the body continues to increase in size, the anterior and posterior ciliated bands become connected about the edges of the ventral depression, and the postero-lateral (?) arms begin to push out laterally from the body. A short medio-dorsal rod may be seen developing in the skeletal system (fig. 6).

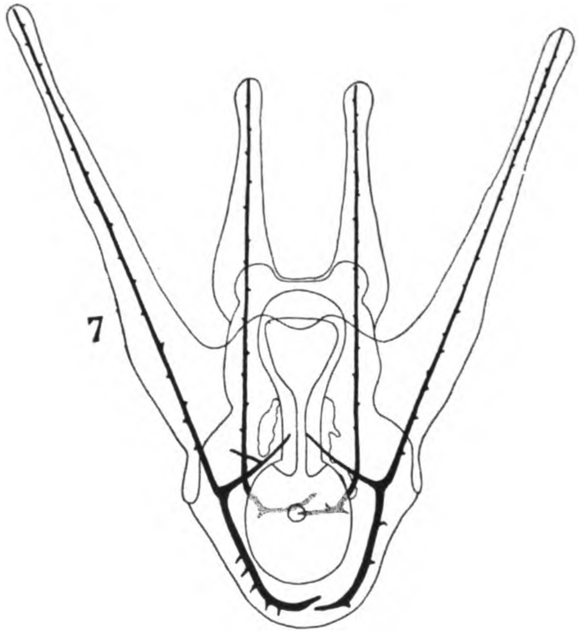


FIG. 7.—*Toxopneustes* pluteus. 6 days.

A brief inspection of a figure of a *Toxopneustes* pluteus of the same age, 6 days (fig. 7), is sufficient to convince one of the striking difference in form. It is evident from a comparison of the development of these two forms that if a cross between the two were possible valuable data might be afforded.

THE CIDARIS FEMALE BY TOXOPNEUSTES MALE CROSS.

The cross is easily made, the *Cidaris* eggs requiring no treatment in order that fertilization may be accomplished. The eggs may be fertilized with *Toxopneustes* sperm immediately after their removal from the ovary, but as a check against the possibility of chance fertilization with *Cidaris* sperm they were kept for 2 hours before inseminating with the foreign sperm. More than 90 per cent of the eggs fertilized; the typical fertilization membrane appeared shortly after the addition of the sperm. In this cross no difference from the characteristic normal *Cidaris* development was noted before the beginning gastrula stage. The fertilization-cleavage interval was not lessened, as in the case of certain other echinoid crosses that I have described. In point of time the appearance of the mesenchyme seemed slightly hastened, although the gain in time is not great enough to warrant a general conclusion, since it is quite within the range of variation between different lots of straight-fertilized eggs. My statement regarding a possible slight hastening refers to comparative straight and cross fertilization in the same lot of eggs. Even here I prefer to draw no conclusions, since I have made the comparison with only eight lots of eggs. As to the *place* of mesenchyme formation there is no chance for either personal equation in the observer or individual variation in the larvæ to play a part.

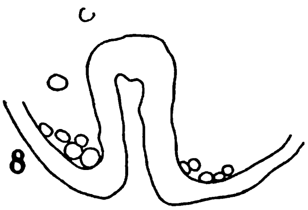
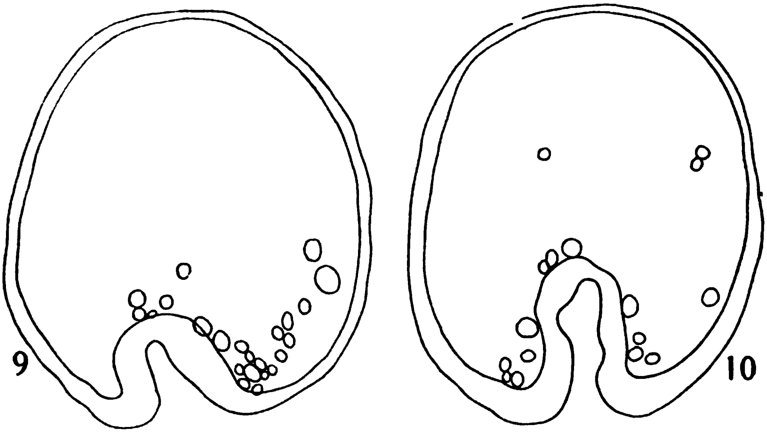


FIG. 8.—Optical section of portion of *Toxopneustes* ♂ × *Cidaris* ♀ gastrula, showing primary mesenchyme cells at base of archenteron. 24 hours.



FIGS. 9 and 10.—Optical section of *Toxopneustes* ♂ × *Cidaris* ♀ gastrula, showing primary mesenchyme cells. 24 hours.

In the hybrids the mesenchyme arises from the sides and around the base of the archenteron, close to its point of union with the blastopore (figs. 8, 9, 10). This was true in every instance. The difference between the normal and the hybrid was so striking that it needed no confirmation by other observers, but purely as a matter of personal interest I asked four other investigators at the laboratory to look at the two sets of living larvæ and describe their observations to me. The independent observation of each of these investigators was a confirmation of my own.

The succeeding stages show considerable variation. In some the growth of the archenteron ceases and the blastocœle becomes filled with a mass of opaque cells (fig. 11). In others gastrulation continues. In a few cases a small skeletal spicule was formed in the mass of mesenchyme cells at the base of the archenteron. I was unable to keep the hybrid material alive beyond the gastrula stage.

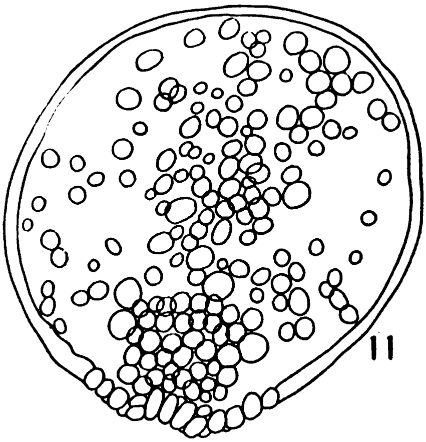


FIG. 11.—Optical section of *Toxopneustes* ♂ × *Cidaris* ♀ abnormal gastrula; blastocœle nearly filled with opaque cells. 40 hours.

GENERAL DISCUSSION.

We have already noted Boveri's conclusions regarding the early influence of the sperm on development. My results confirm his general thesis as to an earlier influence than that commonly accepted, although my conclusion is based on evidence not afforded by his crosses.

In the nature of the case the results given by inseminations in which the sperm nucleus does not unite with the egg nucleus are without direct value in this connection. They may have an indirect value in showing that the sperm cytoplasm, if it has become a part of the egg cytoplasm, has not influenced the early development. As has already been indicated, the larvæ obtained from such inseminations are essentially parthenogenetic.

The only investigation of which I know, which is closely comparable to mine, is that of Godlewski (1907), and even here we are comparing the reciprocal cross between forms whose early development is similar. In Godlewski's cross the fertilization of echinoid eggs (primary mesenchyme formed in the blastula stage) with crinoid sperm (crinoid mesenchyme formed in the gastrula) resulted in larvæ which followed the maternal course of development; this was true even for the fertilized enucleated egg fragment.

In my own work a cross made on material with similar differences of development, namely, the *Cidaris* egg (mesenchyme formed in the gastrula), with *Toxopneustes* or *Hipponoë* sperm (echinoids in which the primary mesenchyme is formed before gastrulation), gave larvæ in which the mesenchyme was formed at the beginning of gastrulation, the mesenchyme arising at the sides and base of the archenteron.

We may ask this question: Is the foreign sperm better able to hasten than to retard certain characteristic processes? It is not now possible to answer this question, but it is one which should incite investigation.

The objection may be raised that as I was unable to keep the hybrid larvæ alive beyond the gastrula stage, the cells which I observed passing into the blastocœle were abnormal and indicated an abnormal condition of the larvæ. Certainly they were not normal *Cidaris* larvæ; they were hybrids, and as hybrids they were normal. The larvæ were not the formless aggregations of cells obtained in some crosses, but proceeded on a perfectly regular course of development. Even granting that when compared with larvæ derived from straight fertilized *Cidaris* eggs, they were not normal, it must be admitted that the abnormality was in the direction of normal *Toxopneustes* mesenchyme formation and also that the condition was brought about by the use of the foreign sperm and that the result indicates that the sperm is able to exert an influence on the character of development at a stage earlier than that heretofore demonstrated.

Shearer, De Morgan, and Fuchs (1911), as well as some other recent workers on the hybridization of echinoids, have come to the conclusion that the early larvæ are of too variable a nature to afford any definite evidence of parental influence. In order that I may not be misunderstood, let me quote directly from the paper mentioned:

> As the result of extensive investigation of the early larval history of our various crosses, we have come to the conclusion that these are too variable to afford any definite evidence of parental influence and especially is this true with regard to the skeleton, heretofore considered the chief index of inheritance.

This conclusion was reached after the investigation of three species of *Echinus*. The authors make the statement with regard to the forms upon which they have worked and do not apply it to all cases. The investigation of a sufficient number of properly chosen forms will convince any investigator that parental influence may be indicated even in early larval stages, and especially is this true with regard to the skeleton.

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IX.

STUDIES OF JAMAICA ECHINI.

BY ROBERT TRACY JACKSON.

Twenty-one figures, including one plate.

STUDIES OF JAMAICA ECHINI.

BY ROBERT TRACY JACKSON.

It was my privilege to join the Carnegie Institution of Washington expedition to Jamaica in the spring of 1912. While there, studies were made of the later stages of development and the variation of a number of species of regular Echini. All the material studied was collected at Montego Bay, close along shore in shallow water of less than a fathom in depth. This locality is very favorable for studying these animals, as abundant material can be obtained, on the reefs or grass-covered bottom, by wading in water usually less than 3 feet in depth. The observations are in extension of those recently published in the "Phylogeny of the Echini" (Memoirs Boston Society of Natural History, vol. 7, 1912). As only a few species are involved, they are taken up systematically, giving under each the results of the observations.

EUCIDARIS TRIBULOIDES (Lamarck).

This species is fairly common at Montego Bay, so that sufficient material was available for laboratory work, but it was not so abundant as the other species of regular Echini studied. It occurs in shallow water and is surprisingly dull and sluggish, presenting none of the activity of motion seen in *Centrechinus* or the more passive *Tripneustes*. In collecting *Eucidaris*, one simply picks them up, and in no case observed did the animal cling to the ground by its tube-feet. The adults at Montego Bay are rather small for the species, not attaining the size of specimens seen from the Bahamas. An average adult from Montego Bay measures about 30 to 35 mm. in diameter. Material from Port Antonio, Jamaica, also is small, but is very abundant, as I had over 500 specimens collected at that locality. Of a limited number of specimens of this species from the Bahamas, the largest noted is 52 mm. in diameter. Only a few young specimens of *Eucidaris* were found at Montego Bay. In individuals measuring up to 10 mm. in diameter the ocular plates are all exsert and the genital pores are wanting. There was not sufficient young material to study the periods at which the ocular plates travel in and reach the periproct. In the adult of this species, typically (58 per cent), oculars V, I, IV are insert, as previously ascertained from an examination of 849 specimens from several localities. A specimen measuring 11 mm. in diameter has all five genital pores in place, as have also all larger specimens. Whether the genital pores would come in at this exact period of growth could only be known from observations on a larger series of specimens than was available. In living specimens, opened when perfectly fresh, it was found that the teeth extend only very slightly above the base of the foramen magnum. As this structure is very shallow in *Eucidaris*, the dorsal border of the tooth very nearly coincides with the upper limits of the lantern, but the tooth does not extend horizontally over the top of the lantern as in *Strongylocentrotus* (Phylogeny of the Echini, p. 179, plate 5, figs. 1, 6). The dental capsules, which are the fleshy bags inclosing the base of the teeth, are small in *Eucidaris*, not large and inflated as they are in *Strongylocentrotus* and in the Echinidæ.

The Cidaridæ, the sole family representing the order Cidaroida, is the only group of modern Echini that extends back into the Palæozoic. The Palæozoic species, which belong to the genus *Miocidaris*, present only slight differences from recent typical members of the order. As an ancient group that has suffered comparatively little change in its whole geological history the structure and development of the Cidaridæ is of especial interest. Something is known of the post-embryonic development of cidarids, due principally to Loven's critical study of young *Goniocidaris* (Echinologica, Stockholm, 1892), but further knowledge of the developing stages of this primitive archaic type is much to be desired.

CENTRECHINUS SETOSUS (LESKE).

The most abundant sea-urchin at Montego Bay is *Centrechinus*[1] *setosus* (Leske), which abounds in countless profusion in shallow water on the reefs. In strong distinction from the quiescent habits of *Eucidaris*, *Centrechinus* is a most active animal, moving about freely and actively and waving its long spines in a threatening fashion. The spines are so sharp and cause such poisonous wounds that they are a constant menace to bathers, and specimens have to be collected with caution or serious results will follow. The tube-feet have only the slightest hold on the sea-floor, so that specimens can be picked up with long-handled forceps without any perceptible resistance. In this *Centrechinus* differs markedly from *Tripneustes*, *Strongylocentrotus*, or *Echinometra*, which on a rocky surface cling tenaciously by the tube-feet. Young specimens of *Centrechinus* have spines which are strongly banded; white and very dark, nearly black, alternating. The light and dark bands are of about equal width. The spines are long in young as well as in adults, and a specimen 12 mm. in diameter has spines 36 mm. in length. A specimen 60 mm. in diameter has spines up to 150 mm. in length. From this it occurs that a small specimen looks rather big with spines extending radially, and a large specimen is menacing indeed when the needle-like sharpness of these weapons is considered. Banded spines as a character are retained until individuals are about 20 to 25 mm. in diameter, after which they are typically a deep uniform black. As an exception, a specimen 40 mm. in diameter was seen, in which all the spines were banded as in youth. Frequently in immature specimens the older ventral spines are banded when the dorsal spines, situated on the later-added and therefore younger plates are quite black. In adults, no case of banded spines was seen, but occasionally pure white spines are interspersed with the black, and in a few cases observed all the primary spines were white in the adult. Whatever the color of the spines, the test is of a dark, uniform black in life.

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[1] *Centrechinus* is a name which I gave (Phylogeny of the Echini, p. 27) to replace the name *Diadema*, which in post-Linnæan usage is preoccupied for a crustacean. To replace an old, established name is unfortunate, and some objection has been raised to my action. As *Diadema* has been in current use as a generic name for sea-urchins, Gastropoda, and Lepidoptera, it seems that the rule of priority must be maintained and a new generic name substituted for *Diadema* in these three groups.

In the family of the Centrechinidæ, *Centrostephanus longispinus* (Phillipi), from the Mediterranean, in adults has spines which are banded white and light brown, the pattern recalling those of young *Centrechinus*. In *Echinothrix calamaris* (Leske), from the Pacific, the spines are very variable in adults, being all dark, purple or green, all white, or banded; great diversity may occur in the spines of one individual. In other families of Echini, banded spines occur occasionally as species characters; they are a feature of *Cœlopleurus maculatus* A. Agassiz and Clark, and are common in species of the Temnopleuridæ.

In my previous studies of ocular plates and their variation in this species, there was a limited number (110) of developing specimens, and of these very few were really small. Of adults, 1,168 were examined from various localities. With a good series of developing specimens from a single locality, certain differences are brought out from the earlier limited observations. There are also differences in the percentages of adult characters from what obtained in the species as a whole from different localities. In table 2 (page 156) are given the characters of developing series, and the variations of adults of this and other species are considered. The aberrant variations are given in detail only under the consideration of the species in the text.

As to the developing series of *Centrechinus setosus*, in 50 specimens from 4 to 15 mm. in diameter, all the oculars are exsert (fig. 1). In 103 specimens 15 to 20 mm. in diameter, 78 per cent have all oculars exsert, 4 per cent have ocular I insert, and 5 per cent have ocular V insert. In 2 per cent oculars I, V are insert, and in 7 per cent oculars I, V, IV have reached the periproct. Of aberrant variants of this size there are 5 per cent, one specimen having ocular IV only insert; one having I, IV insert; two having V, IV insert; and one has oculars I, V, IV, III insert. It is interesting to see that at this youthful period, while a high percentage have oculars all exsert, many stages of development as regards the oculars are presented by some of the specimens. Of the next larger size, 20 to 25 mm., 270 specimens, 51 per cent still have all the oculars exsert; 1 per cent have ocular I insert; and 16 per cent have ocular V insert; 11 per cent have oculars I, V, and 17 per cent have oculars I, V, IV insert. In one specimen, 0.4 per cent, oculars I, V, IV, II are insert. There are eight aberrants, or 3 per cent; of these, 1 has ocular IV insert; 1 has I, IV, and 6 have oculars V, IV insert.

In the next larger series, 25 to 30 mm. diameter (240 specimens), 21 per cent have all oculars exsert; 3 per cent have ocular I, and 8 per cent have ocular V alone insert; 14 per cent have oculars I, V, and 45 per cent have oculars, I, V, IV insert. As this last is the species character, it is of note that this is the first stage at which this species feature has become a dominant character. In 1 per cent, oculars I, V, IV, II, and in 1 per cent all oculars are insert. Seventeen specimens, or 7 per cent, have an aberrant arrangement. Of these, 4 have ocular IV only insert; 11 have oculars V, IV, one has I, V, II, and one has oculars I, V, IV, III insert. The occurrence of I, V, II insert as an aberrant variant is of interest because this, as previously shown, is a common aberrant when three oculars reach the periproct in the Echinidæ and Strongylocentrotidæ, and occurs also in the Stomopneustidæ. I have not found a case of I, V, II insert in the Cidaridæ, and it is the only case seen in *Centrechinus setosus*, although in this species, 1,398 cases of I, V, IV insert have been observed.

In the next series of *Centrechinus setosus*, 30 to 35 mm. diameter, 245 specimens, 10 per cent have all oculars exsert; 2 per cent have I only; and 12 per cent have V only insert; 5 per cent have I, V, and 63 per cent have I, V, IV insert; 4 per cent have I, V, IV, II insert. Of aberrants, there are 4 per cent, these consisting of 8 specimens with oculars V, IV insert, and 2 with oculars I, V, IV, III insert.

The next series, measuring 35 to 40 mm. in diameter, 169 specimens, has 5 per cent with all oculars exsert; 2 per cent with I only; and 3 per cent with V only insert; 5 per cent have I, V, and 71 per cent have I, V, IV insert; 6 per cent have I, V, IV, II, and 2 per cent have all oculars insert. Aberrants are 5 per cent; of these, two specimens have ocular IV alone insert; 1 has I, IV; 4 have V, IV; and 2 have I, V, IV, III insert.

The series 40 to 50 mm. in diameter is considered the last of the developing series. Of this size, 162 specimens, 4 per cent have all oculars exsert, 1 per cent have ocular I, and 0.6 per cent have ocular V only insert; 6 per cent have oculars I, V, and 70 per cent I, V, IV insert; 9 per cent have oculars I, V, IV, II, and 6 per cent have all oculars insert. Of aberrants there are 3 per cent, one specimen having V, IV and 4 having I, V, IV, III insert. The series 35 to 40 mm. and 40 to 50 mm. in diameter make a close approach to the mature series as regards the species character of I, V, IV insert, but these two younger series have more of the developing and fewer of the progressive characters than are seen in the older series.

While *Centrechinus setosus* is profusely abundant at Montego Bay, specimens do not attain as large a size as in Florida, from which locality many individuals exceed 100 mm. in size. Of the adult series from Montego Bay, 50 to 80 mm. in diameter, 162 specimens, one specimen, or 0.6 per cent, had ocular V only, and one had oculars I, V insert, both of these specimens are between 50 and 60 mm. in diameter; 73 per cent have I, V, IV insert, the species character; 13 per cent are progressive variants with I, V, IV, II insert and 10 per cent are extreme progressive variants with all oculars insert; 3 per cent are aberrant variants, all five specimens having oculars I, V, IV, III insert.

Considering the ocular-plate arrangement in *Centrechinus* from Montego Bay as a whole, it is seen, as shown clearly in table 2 (page 156), that from all exsert,the character of the young, there is a steady progressive series with growth in the development or traveling in of ocular plates. *Centrechinus* is a member of the most primitive suborder, the Aulodonta, of the Centrechinoida, and as such is worthy of special attention, for, as shown by Professor Hyatt, primitive types in a group have a slow development as compared with specialized types, and in character approach nearest to the next lower series of their own phylum. The development of *Centrechinus* amply bears out this important truth.

Of the total 1,401 specimens of *Centrechinus setosus* listed from Montego Bay, the aberrants are 59 in number, of which 8 have ocular IV only insert; 3 have oculars I, IV insert; 32 have oculars V, IV insert; 1 has oculars I, V, II insert, and 15 have oculars I, V, IV, III insert. It is noteworthy that in the mature series (50 to 80 mm. diameter) the only aberrants that occurred were cases of oculars I, V, IV, III insert, thus indicating that the other aberrants seen in immature individuals may be considered as irregularities in the order of sequence of the coming in of ocular plates, rather than as aberrants that would have retained the given character if they had lived to grow up.

In specimens of *Centrechinus* up to 14 mm. in diameter (48 individuals), all the genital plates are imperforate (fig. 1), whereas in those 14.5 mm. and all larger with very few exceptions, all genital plates have the genital pores.[1] In comparison with this, the specialized *Strongylocentrotus dröbachiensis* attains its genital pores when between 5 and 10 mm. in diameter (Phylogeny of the Echini, pp. 131, 170). *Centrechinus* has all oculars exsert up to 15 mm. diameter, and with intermediate stages still has 4 per cent all exsert when 40 to 50 mm. diameter. *Strongylocentrotus dröbachiensis*, on the contrary, has typically oculars all exsert only in very young specimens and has only 29 per cent with all exsert in specimens (51) between 2.5 and 4 mm. in diameter; further, it has only 4 per cent with oculars all exsert in specimens (82) between 4 and 5 mm. diameter. *Centrechinus* first attains the species character of oculars I, V, IV insert as a dominant feature (45 per cent) when it is 25 to 30 mm. in diameter. *Strongylocentrotus dröbachiensis* attains I, V insert, the species character, as a dominant feature (52 per cent) when 5 to 10 mm. in diameter. In *Centre-*

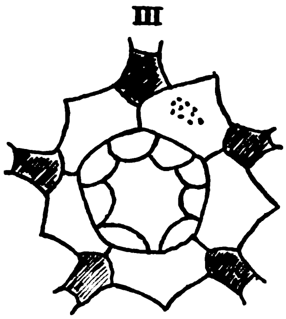

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FIG. 1.—*Centrechinus setosus* (Leske). Montego Bay, Jamaica. × 12. Diameter 11 mm. Oculars all exsert. No genital pores, large marginal plates in periproct. All these are youthful characters.

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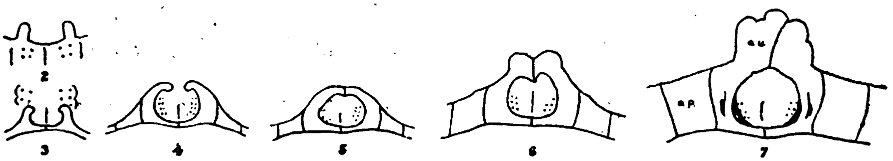
[1] I regret to say that by error in the figure published of a young *Centrechinus* (Phylogeny of the Echini, fig. 88, p. 106), genital pores are shown. The specimen has no pores and they were inadvertently drawn.

chinus when oculars begin to enter the periproct, the first to enter is V or I, but in the Montego Bay series V is by far the more common,[1] they being in the ratio of 102 of ocular V to 23 of ocular I. In this feature *Centrechinus* makes an approach to the Cidaridæ, in which group, when one ocular is insert, it is almost always ocular V. As previously shown, when in the Centrechinoida four oculars reach the periproct, it is typically I, V, IV, II, and when any other combination exists it is nearly always I, V, IV, III. This combination, while rare in the Centrechinoida as a whole, is relatively more frequent in *Centrechinus* and is a typical feature or a frequent variant in some species of the Cidaridæ. In this feature also, therefore, *Centrechinus* makes a certain approach to the Cidaroida.

In *Centrechinus*, when opened fresh and alive, it is found that the teeth extend above the base of the foramen magnum about to the upper line of the lantern, but the proximal base of the teeth does not extend horizontally over the lantern as in the Echinidæ, Strongylocentrotidæ, and Echinometridæ. Further, the dental capsule, inclosing the base of the tooth, is small. In the limits of the teeth dorsally and the small dental capsule, as well as in the grooved teeth, *Centrechinus* makes a close approach to the characters of the Cidaridæ as represented by *Eucidaris tribuloides*.



FIGS. 2–6.—Perignathic girdle in *Aspidodiadema* and its development in *Centrechinus*.

2.—Adult *Aspidodiadema meijerei* (Döderlein), Pallolo channel, Hawaiian Islands. The auricles are separate spur-like styles.

3.—*Centrechinus setosus* (Leske), Montego Bay, Jamaica. Specimen 5 mm. diameter. Auricles are separate styles, apophyses faint.

4.—The same. Specimen 10 mm. diameter. Auricles are more developed and arch over the ambulacra.

5.—The same. Specimen 25 mm. diameter. Auricles meet over the ambulacra in a slender arch.

6.—The same. Specimen 70 mm. diameter. Auricles developing a high plate-like character, apophyses also are high.

7.—*Centrechinus setosus*, Bermuda. Specimen 110 mm. diameter. Auricles have developed a high plate-like character; apophyses are high ridges.

Very little attention has been paid to the development of the perignathic girdle excepting by Lovén, who described it in *Cidaris* and *Strongylocentrotus* (Echinologica). I also have published a few observations on the development and variation of the perignathic girdle. *Centrechinus*, while being a primitive type in many respects, has in the adult a specialized perignathic girdle having high auricles which meet in a broad plate-like arch over the ambulacra and also high apophyses on the interambulacral areas (fig. 7). Such being the character of the adult, it is of much interest to follow the development of this, which is one of the most specialized perignathic girdles known in Echini. When a specimen is 5 mm. in diameter, the apophyses show only slight indications of development and the auricles stand up as separate, blunt, spur-like processes on either side of the ambulacrum (fig. 3).

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[1] In previous observations from various localities with limited material, ocular I predominated over V numerically.

The upper ends of the auricles at this early stage are slightly flattened, but do not arch over the ambulacrum at all. In a specimen 10 mm. in diameter, the apophyses have developed somewhat, and the auricles are inclined over the ambulacral area, but do not meet (fig. 4). In specimens 25 mm. in diameter the apophyses have developed into distinct elevated ridges, and the auricles have met over the ambulacral areas as slender arches (fig. 5). From this point on to the adult the development of the perignathic girdle is marked by the increase in height of the apophyses and concurrently the increase in height and the lamellar expansion of the auricles (figs. 6, 7).

In seeking comparisons to these stages of *Centrechinus* in the adults of related forms, it is found in *Aspidodiadema meijerei* (Döderlein), *A. nicobaricum* Döderlein, and *Dermatodiadema horridum* A. Agassiz, representing the family Aspidodiadematidæ, that the auricles exist as small conical spurs situated on either side of the ambulacral areas, and apophyses are not developed (fig. 2). This condition corresponds with the first stage noted in the development of *Centrechinus* (fig. 3). This relation of the perignathic girdle is of much interest because in the Aspidodiadematidæ the simple ambulacral plates, the large primordial ambulacral plates on the peristome, the large apical disk and simple spur-like auricles are all primitive characters, indicating that the family is more primitive structurally than is the still primitive family of the Centrechinidæ. While primitive in many characters, the Aspidodiadematidæ is specialized in that all the oculars reach the periproct, and *Centrechinus*, while belonging to the primitive suborder Aulodonta, is specialized in its great development of the perignathic girdle. This shows how primitive and specialized features may be combined in one and the same type.

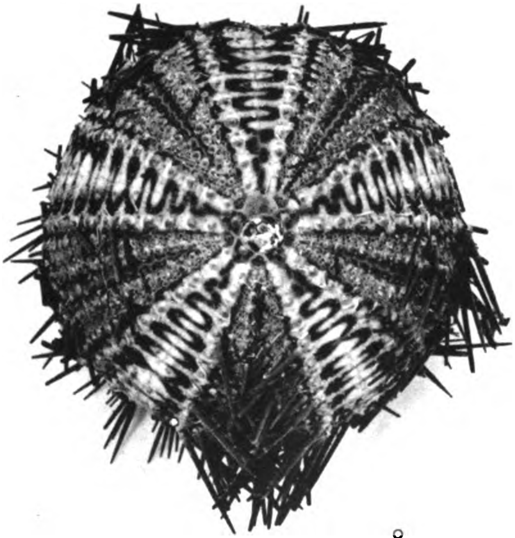
Mr. Agassiz (Panamic Echini, 1904, p. 59) says of the Aspidodiadematidæ: "The auricles are most irregularly developed. They are either wanting or mere projections, slightly raised." I have examined three representative species and a number of specimens of this family, and find no evidence that the auricles are irregularly developed. In adults they are not wanting except where broken off. They are perfectly definite spur-like projections, and of much interest as being structurally the most primitive auricles known in any of the adult Centrechinoida. As the Cidaroida have apophyses but no auricles, and as these structures make their first known appearance in the Centrechinoida, the simplicity of their structure in adults of the Aspidodiadematidæ and in the young of *Centrechinus* is of particular interest.

TOXOPNEUSTES VARIEGATUS (Lamarck).

A common species at Montego Bay is *Toxopneustes variegatus* (Lamarck), which is found abundantly on the grass-covered bottom in shallow water. Specimens attain a large size, some measuring 70 mm. in diameter. Few young specimens were found, though this is perhaps due to the fact that special search was not attempted, from limitations of time. The test is mottled green and white, with green spines as the prevailing color. Some young specimens show on the green and white groundwork purple primary tubercles and purplish spines. A peculiar color-pattern was observed as a relatively rare variation in the fact that in the interambulacral areas of some specimens the green followed a zigzag pattern on a white ground and extended thus from the apical disk adorally, but in no case observed extended further than the ambitus. The zigzag green color-pattern on a cream-white ground of a specimen measuring 45 mm. in diameter is shown in fig. 8. The lower figure on the same plate is of a somewhat larger specimen, measuring 48 mm. in diameter, in which the zigzag pattern occurs only near the apical portion of the test. Both of these specimens with others figured in this paper are now in the collections of the Museum of Comparative Zoölogy, at Cambridge, Mass. This type of color-pattern was quite new to Dr. Clark and Professor Tennent, both of whom had worked on the species, and I had never seen it before, although I have studied many specimens from different localities.

Being busy with other species, no attempt was made to collect a large series of *Toxopneustes variegatus* for a study of ocular plates, but 400 specimens are tabulated. These are of various sizes, but none were young and they were not graded into sizes for comparison. Of this series, 2 specimens, 0.5 per cent, have ocular I only insert as arrested variants, and one specimen, 0.3 per cent, has ocular V only insert; 84 per cent have oculars I, V insert (figs. 8, 9), which is the species character in all localities; 15 per cent are progressive variants with oculars I, V, IV insert. Four specimens, 1 per cent, are aberrant variants and all of these have oculars I, V, II insert. The ocular character is practically the same as I showed previously in this species from several localities, but the Montego Bay material has a somewhat lower percentage of I, V and corresponding higher percentage of I, V, IV insert. My previous observations on 1,043 specimens gave 90 per cent I, V insert and 8 per cent I, V, IV. Comparison of the tabulation of this species may be made with that of the closely allied *Toxopneustes atlanticus* (A. Agassiz) from Bermuda, in which I showed (Phylogeny of the Echini, p. 161) that the mature series (45–77 mm. diameter) has a much higher percentage (28 to 29 per cent) of oculars I, V, IV insert than has *Toxopneustes variegatus*. The Montego Bay experience in the cases of several species brought out the fact clearly that for a close study of variation it is very desirable to tabulate ocular plate characters in specimens from a single definite locality because considerable difference in a given species may occur in different localities.

In *Toxopneustes variegatus*, when opened fresh, it is found that the proximal part of the teeth extend horizontally over the top of the lantern and the teeth are bent back on themselves in the same plane, also the dental capsules which inclose the base of the teeth are large and bladder-like, in these characters differing from *Eucidaris* and *Centrechinus*, but agreeing with *Strongylocentrotus* as I previously described it. In a young specimen 12 mm. in diameter, the auricles are still separate, but in larger specimens they are



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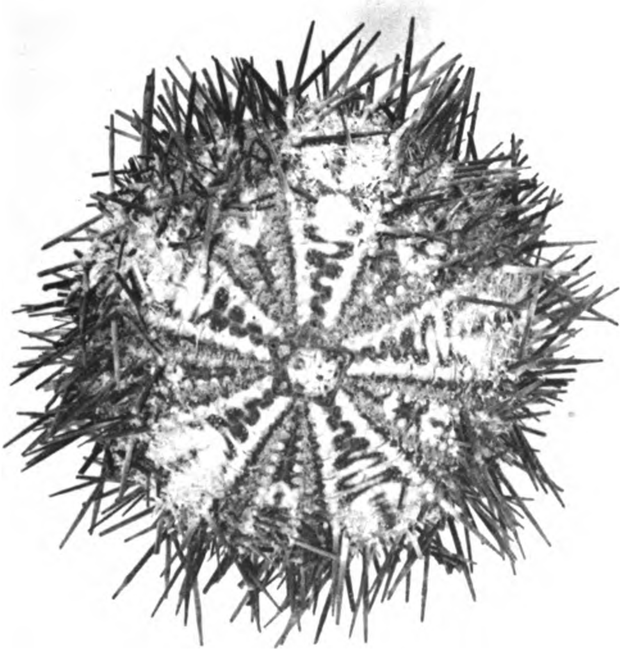

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joined in an arch over the ambulacral areas. In large adults the apophyses are developed as a moderate ridge and auricles are joined in a delicate arch which never becomes heavy, as in large specimens of *Tripneustes*.

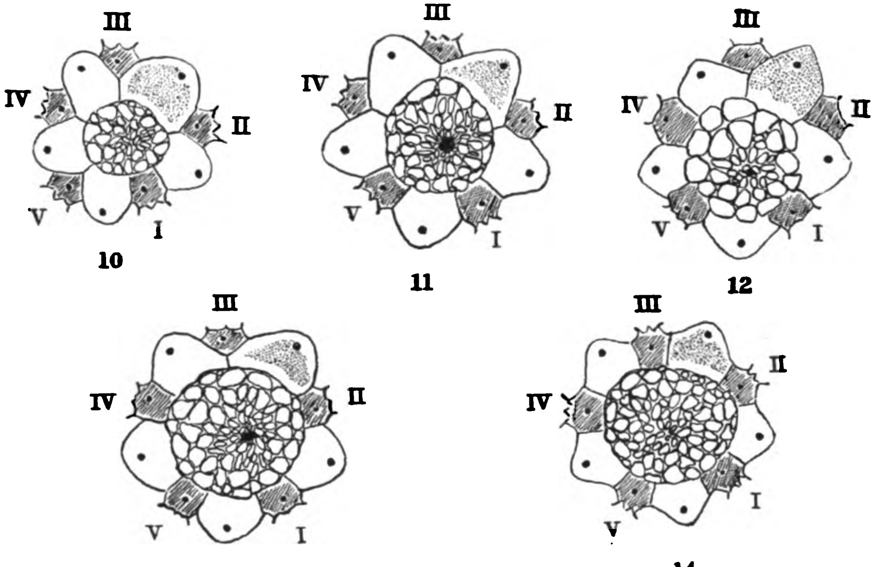
TRIPNEUSTES ESCULENTUS (Leske).

One of the abundant species at Montego Bay is *Tripneustes esculentus* (Leske). It occurs on grass bottom and also on the reefs in shoal water, and is a strikingly handsome sea-urchin when alive, with its white spines against the dark purplish groundwork of the fleshy covering of the test. The larger individuals live out in the open in full view, but young specimens are found only in crevices or on the under side of rocks. The young are not common, and although sought for diligently, no very young specimens were found and only a limited number of the youngest seen were obtained. Perhaps some other time of year, as summer or autumn, would be more favorable for young material of this species. In *Tripneustes esculentus* from this locality the ocular plates continued to travel in or enter the periproct, from the youngest to the largest series of specimens observed, as shown by the tabulation. For this reason, as regards the feature of ocular plate development, what is called the developing series for this species in the table is arbitrarily drawn at a maximum of 70 mm.

Taking up the developing series: Ten specimens of 20 to 25 mm. diameter have 50 per cent with ocular I only insert, and 50 per cent with oculars I, V insert. Younger specimens would doubtless have all oculars exsert as a developing stage. The next size, 25 to 30 mm. diameter, 21 specimens, has 24 per cent with ocular I only insert and 76 per cent with I, V insert. The relations of these two younger series shows that it is a period of rapid development. The next series, 30 to 40 mm. diameter, 60 specimens, has 12 per cent with I only insert; 80 per cent with I, V insert; 2 per cent with I, V, IV and 2 per cent with I, V, IV, II insert. Three specimens, 5 per cent, are aberrant with I, V, II insert. This stage, while holding the I only insert as a developing character, has attained the I, V; the I, V, IV; and the I, V, IV, II insert; which are each respectively the feature of the species as a character in one or more of the several localities from which material was studied, but the relative proportions of the three characters differ from that of any known adult series.

The series 40 to 50 mm. diameter, 42 specimens, has lost the I insert as a youthful character; 90 per cent have oculars I, V insert; 2 per cent have I, V, IV, and 2 per cent have I, V, IV, II insert. Five per cent are aberrant, one of the two specimens having I, V, II insert and the other V, II insert. This stage has the highest percentage of I, V insert of any age of material from Montego Bay. The series 50 to 70 mm. diameter, 34 specimens, has 53 per cent with oculars I, V insert; 18 per cent with I, V, IV; and 6 per cent with I, V, IV, II insert. Aberrants are 24 per cent, eight specimens, all of which have oculars I, V, II insert. The developing series in its later phases may be compared broadly with material from Bermuda or other localities for the species in which oculars I, V insert is the adult local character, as it is the youthful character in these immature stages from Montego Bay.

The next larger series, 70 to 90 mm. diameter, 125 specimens, has one specimen with ocular I insert as an arrested variant; 30 per cent have oculars I, V insert. The latter, which was the leading character in the later developing stages, has now passed into the phase of an arrested variant. 34 per cent have oculars I, V, IV insert, and this is the dominant character of this age. As a phase it can be compared with a series of adults from the Bahamas in which locality I, V, IV insert is the typical character. Twenty-eight per cent have oculars I, V, IV, II insert, which at this stage may be considered a progressive character as it is at all other known localities for the species. In 8 per cent the ocular arrangement is aberrant, all of the ten specimens having oculars I, V, II insert.

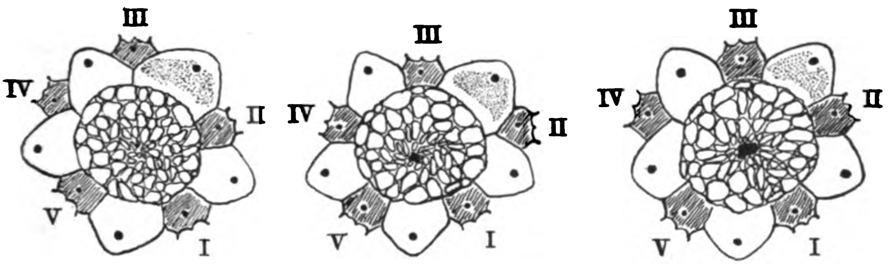


10 11 12 13 14

FIGS. 10–14.—Normal ocular plate arrangement in *Tripneustes esculentus* (Leske), Montego Bay, Jamaica. All figures nearly × 2.

10.—Diameter 99 mm. Ocular I only insert, an extreme arrested variant.
11.—Diameter 114 mm. Oculars I, V insert, an arrested variant for the locality.
12.—Diameter 110 mm. Oculars I, V, IV insert, an arrested variant for the locality.
13.—Diameter 115 mm. Oculars I, V, IV, II insert, the typical character for the locality.
14.—Diameter 106 mm. All oculars insert, a progressive variant.

The next series, 90 to 110 mm. diameter, 200 specimens, has one specimen with ocular I only insert as an arrested variant (fig. 10), and one specimen with ocular V alone insert. For ocular V only to be insert is a rare variation in *Tripneustes* and is unusual in the family Echinidæ. In 22 per cent oculars I, V are insert. This, which was the character of the later developing series and is the character of adults at Bermuda and Florida, has dropped considerably in its percentage as an arrested variant. In 24 per cent oculars I, V, IV are insert. This character for Montego Bay has at this stage dropped into the phase of an arrested variant, though it was the dominant character in the series 70 to 90 mm. in diameter and is the adult character in a series from the Bahamas. Forty per cent have oculars I, V, IV, II insert. This is the first series in which this character is a dominant feature at Montego Bay, and for all earlier stages in this locality and for all other localities known it is a progressive character. Two per cent have all oculars insert as a progressive variant (fig. 14). It is somewhat striking that with four oculars insert as a dominant character, there should not be more specimens with all oculars insert. In this series, 25 specimens, 13 per cent, have an aberrant arrangement of ocular plates. Of these aberrants, 22 have oculars I, V, II insert; two have I, V, IV, III insert (fig. 16), a very rare variant in the Echinidæ when four plates reach the periproct, but although rare here, it is a common character in the Cidaridæ and a not rare variant in *Centrechinus setosus*. One aberrant specimen of *Tripneustes* has oculars I, V, II, III insert (fig. 17). This, which is a right-handed equivalent of I, V, IV, III (just as I, V, II is a right-handed equivalent of I, V, IV) is a unique variant for Echini as far as my experience goes. In the Centrechinoida, as tabulated in my preceding memoir and in the present paper, 619 specimens are recorded, in which four plates reach the periproct. Of these, in 554 specimens (89.5 per cent) it is oculars I, V, IV, II the bivium and posterior pair of the trivium, in 64 specimens (10.3 per cent) it is the aberrant arrangement I, V, IV, III, and in the one specimen cited (0.16 per cent) it is I, V, II, III. (Compare figs. 13, 16, 17.)



15 16 17

FIGS. 15–17.—Aberrant ocular plate arrangement in *Tripneustes esculentus* (Leske), Montego Bay, Jamaica. All figures nearly × 2.

FIG. 15.—Diameter 115 mm. Oculars I, IV, II insert, a common aberrant variant, a right-handed equivalent of the normal character I, V, IV insert.
FIG. 16.—Diameter 106 mm. Oculars I, V, IV, III insert, a rare aberrant variant, but the usual one when four oculars but not I, V, IV, II are insert.
FIG. 17.—Diameter 102 mm. Oculars I, V, II, III insert, a unique aberrant variant, a right-handed equivalent of I, V, IV, III insert.

The series containing the largest specimens, 110 to 132 mm. diameter, 82 specimens, has 13 per cent with oculars I, V insert as arrested variants (fig. 11); 24 per cent with I, V, IV insert as arrested variants (fig. 12); and 43 per cent with I, V, IV, II insert as the local character of the species (fig. 13). Five per cent have all oculars insert as a progressive variation, and 15 per cent, 12 specimens, are aberrant in ocular arrangement. Of these aberrants, 11 specimens have oculars I, V, II insert (fig. 15), and one has V, IV, II insert. In this specimen genitals 5 and 1 are fused, mechanically shutting out ocular I from access to the periproct.

The series of *Tripneustes esculentus* from Montego Bay is very interesting from the point of view of ocular development on several accounts. The specimens show a direct progressive increase in the number of oculars insert and the relative percentages of the same from the youngest to the largest series observed. In specimens fully matured, as regards this character, and including from 90 to 132 mm. in diameter, they have as the dominant character (40 to 43 per cent) the oculars I, V, IV, II insert. This is the only locality known for this species, and it is the only recent species known[1] in which four oculars reach the periproct as a typical character. In previous studies of *Tripneustes esculentus*, many specimens were tabulated from several localities, but without detailed measurements of the size of the specimens. As the material was from various sources, it is impossible to go over it again to ascertain the sizes but it is fair to say that the material was practically all adults. With this qualification it is interesting to compare the tabulations of *Tripneustes esculentus* from the several localities from which there was sufficient material to give any reasonable basis for consideration.

TABLE I.

No. of specimens.	Locality.	Ocular I insert; V, IV, II, III exsert.		Ocular V insert; I, IV, II, III exsert.		Oculars I, V insert; IV, II, III exsert.		Oculars I, V, IV insert; II, III exsert.		Oculars I, V, IV, II insert; III exsert.		All oculars insert.		Arrangement of oculars aberrant.	
		p. ct.	No.	p. ct.	No.	p. ct.	No.	p. ct.	No.	p. ct.	No.	p. ct.	No.	p. ct.	No.
193	Bermuda..	2	3	.....	.....	61	117	35	67	2	3	0.5	1	1	2
112	Florida...	.....	.....	1	1	46	52	29	33	12	13	.....	.....	12	13
160	Bahamas..	.....	.....	0.6	1	22	35	46	73	27	43	2	3	3	5
282	Jamaica[1]..	0.4	1	0.4	1	19	54	24	67	40	114	3	8	13	37

[1] 90 to 123 mm. diameter.

As shown in table 1, *Tripneustes* from Bermuda is characterized strongly, 61 per cent, by having oculars I, V insert, with 35 per cent I, V, IV insert as progressive variants, also in 2 per cent I, V, IV, II, and 0.5 per cent all oculars one insert as progressive variants. The aberrants are few, 1 per cent, one specimen having I, V, II and one having V, IV, II insert. This Bermuda lot is the most primitive of the species that I know of as regards ocular arrangement in adults.

Tripneustes from Florida has 46 per cent with oculars I, V insert, which is still the dominant character, but with a lower percentage than at Bermuda. As progressive variants, 29 per cent have I, V, IV insert, and 12 per cent have I, V, IV, II. The aberrants are 12 per cent, all being cases of I, V, II insert. Florida specimens, while having oculars I, V insert as the character, have a lower percentage of this character and a higher percentage of progressive variants than Bermuda material, hence it is structurally an advance on that and a step towards the next higher advance as represented in the series from the Bahamas.

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[1] In *Acrosalenia pseudodecorata* Cotteau, from the Bathonien of France, oculars I, V, IV, II are said to be insert as a species character (Phylogeny of the Echini, p. 112).

Material from the Bahamas collected by C. J. Maynard has only 22 per cent of I, V insert. This is not the character of the locality and has therefore passed into the phase of an arrested variant. There are 46 per cent with oculars I, V, IV insert. This is the dominant character of the Bahama material, while it would be a progressive variant for Bermuda and Florida, and further, would be an arrested variant for the Montego Bay series. In the Bahamas, 27 per cent have I, V, IV, II insert, a common progressive variant, and 2 per cent have all oculars insert. The aberrants are relatively few, 3 per cent, four of the aberrants having I, V, II insert and one having I, V, IV, III insert. Structurally, in its percentages of ocular arrangement, the Bahama material is intermediate between the Florida and Montego Bay series.

The Montego Bay material, considering only the two largest-sized series tabulated on page 156 and treating them as a single series of 90 to 132 mm. diameter, has 19 per cent with oculars I, V insert as a moderately common arrested variant, but with the lowest percentage of any of the localities given. In 24 per cent oculars I, V, IV are insert, which, for this locality, is an arrested variant, though it is a progressive variant for Bermuda and Florida, and a dominant character for the Bahamas. In 40 per cent, oculars I, V, IV, II are insert, the dominant character for this locality, though it is a progressive variant for all the other localities; 3 per cent have all the oculars insert, which as a progressive variant, is only a slight advance on that existing in the other regions cited. The aberrants, 13 per cent, have already been considered above, and do not need further mention excepting that it is interesting that most of them are cases of I, V, II insert, as in other localities.

In all the localities given *Tripneustes esculentus* is an abundant form, and in all attains a large size. The specimens from Bermuda were collected for me and averaged very large, the largest measuring 145 mm. in diameter, yet this locality has the lowest ocular index. The cause of the progressive evolutionary series as marked by the higher and higher percentages of ocular plates reaching the periproct is unknown, but it is a fact that it exists and points to the desirability of studying large series of specimens from different localities.

In *Tripneustes esculentus*, when opened alive, it is seen that the teeth extend above the top of the lantern and horizontally over the top of the same. The proximal end of the tooth is curved back on itself and is grooved, the keeled character developing as one passes orally along the tooth. The dental capsules are relatively large, all this structure coinciding with that previously described in *Strongylocentrotus dröbachiensis*. In a young specimen of *Tripneustes*, 24 mm. in diameter, the auricles are slender, but already meet in an arch over the ambulacra. In large adults the auricles are quite massive and are fused over the ambulacra in a long median symphysis. They may attain a height of 14 mm. or more. The apophyses are also relatively high and massive in large specimens.

ECHINOMETRA LUCUNTER (Linné).

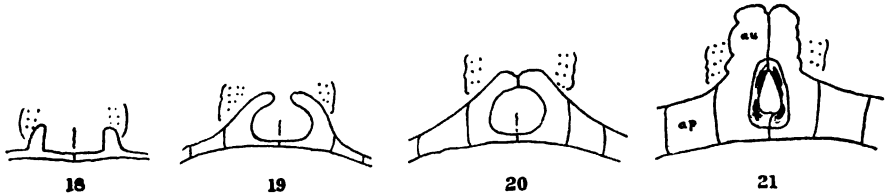
Close in to shore on the coral rock at Montego Bay and in very shallow water *Echinometra lucunter* (Linné) occurs in abundance. Not only adults but individuals of all sizes, down to very young, live in the same exposed situation, where they get the full beat of the waves on shore. This species clings tenaciously to the rock and has to be pulled off from the rock with some effort. The ocular development of this species is very interesting, but unfortunately this was not appreciated until it was too late to gather more material and only a limited series was collected. These were supplemented by a few specimens collected at the same time by Mr. G. M. Gray. A large series could easily be obtained in this locality. As *Echinometra lucunter* is more or less elliptical in horizontal outline, being elongated in the plane of the axis of interambulacrum 3 and ambulacrum I, the measurements of sizes are given in length instead of in diameter, as in the other species considered.

Considering the developing series of *Echinometra*, a set of 34 specimens measuring 4.5 to 15 mm. in length have all the oculars exsert. Of this series, those from 4.5 to 10 mm. in length (26 specimens) were all wanting in genital pores, but these pores exist in all the larger sizes examined. The series 15 to 20 mm. in length, 23 specimens, has 91 per cent with all oculars exsert and 9 per cent with ocular V insert. While ocular I is the first to enter the periproct in the Echinidæ and Strongylocentrotidæ, it is ocular V that typically enters the periproct first in the Arbaciidæ and the Echinometridæ, thus indicating family distinctions in ocular development.

In the series 20 to 25 mm. in length, 58 specimens, in 71 per cent oculars are all exsert and 29 per cent have ocular V insert. In the 25 to 30 mm. series, 110 specimens, 34 per cent have oculars all exsert; 2 per cent have ocular I insert. These are the only cases with ocular I only insert found in Montego Bay material and it is a rare variation in the genus and family, as previously shown. In 54 per cent ocular V is insert. This series is the first stage in growth in which the typical species character of V insert is a dominant feature. In 10 per cent, oculars V, I are insert and in the limited number of specimens this is the first stage at which this character appeared. With more material some specimens would probably be found having I, V insert at an earlier stage of growth. This series is considered the last of the developing stages, but the line as regards ocular development is arbitrary because, as seen in the later series, oculars continue to come in with increasing size of the individuals.

Of the series 30 to 40 mm. in length, 175 specimens, 17 per cent have oculars all exsert as arrested variants, 58 per cent have ocular V alone insert as the typical species character, and 25 per cent are progressive variants with oculars V, I insert. The percentage of V insert at this age is quite near that of the next larger series, but there are more arrested and fewer progressive variants than in the older series. The series 40 to 52 mm. in length, 103 specimens, includes the largest specimens collected at Montego Bay. From other localities specimens may attain a length of 90 or more millimeters. Of this series, 7 per cent have all oculars exsert as arrested variants; 66 per cent have ocular V insert as the maximum development of the species character in the locality; 26 per cent have oculars V, I insert as progressive variants. One specimen, 1 per cent, has oculars V, II insert as an aberrant variant, and this was the only aberrant found in the whole series of 503 specimens collected at Montego Bay.

Considering the ocular arrangement of *Echinometra lucunter* at Montego Bay as a whole: It is a desirable type to study because its species character is to have one ocular insert, and this is a very unusual feature in Echini, otherwise occurring only in the recent Saleniidæ and in *Echinus magellanicus* Philippi from South America. Other Echini, both recent and fossil, are characterized as the species feature of adults by having all oculars exsert, or two oculars insert, or three or more insert, usually three or five. Five insert is a not unusual feature in both living and fossil types as far back as the Palæozoic. In *Echinometra lucunter* it is seen that oculars are all exsert up to a comparatively large size, 15 mm., after which oculars travel in gradually with increasing size, the highest percentages of insertness being in full-grown specimens. Comparing the present tabulation of this species with that previously published (Phylogeny of the Echini, p. 163), it is seen in both that ocular V insert is the species character, though in Bermuda material oculars V, I insert is nearly as frequent. In both lots ocular I insert is a rare feature, and aberrant ocular arrangement is rare. As material previously tabulated was not graded by size, no comparison can be made in this respect.



FIGS. 18–21.—Development of the perignathic girdle in *Echinometra lucunter* (Linné).

FIG. 18.—Young specimen from Montego Bay, Jamaica. Specimen 5 mm. long. Auricles are erect separate styles.

FIG. 19.—The same. Specimen 8 mm. long. Auricles arched over the ambulacrum but separate, apophyses developing.

FIG. 20.—The same. Specimen 15 mm. long. Auricles joined in suture over the ambulacrum.

FIG. 21.—Large adult from Bermuda. Specimen 65 mm. long. Auricles produced vertically as high spoon-like plates, apophyses high ridges.

The teeth of *Echinometra lucunter* extend horizontally over the top of the lantern and the proximal end of the tooth is bent back on itself and in the same plane, as previously described in *Strongylocentrotus*. The proximal part of the tooth is grooved, the keel developing as one passes adorally;

TABLE 2.—*Typical ocular plate arrangement and variation of Echini from Montego Bay, Jamaica.*

Total No. of specimens.	Species and series.	Specimens observed.	Size of specimens.	All oculars exsert.		Ocular I insert; V, IV, II, III exsert.		Ocular V insert; I, IV, II, III exsert.		Oculars I, V insert; IV, II, III exsert.		Oculars I, V, IV insert; II, III exsert.		Oculars I, V, IV, II insert; III exsert.		All oculars insert.		Arrangement of oculars aberrant.	
				p. ct.	No.	p. ct.	No.	p. ct.	No.	p. ct.	No.	p. ct.	No.	p. ct.	No.	p. ct.	No.	p. ct.	No.
			mm. Diam.																
	Centrechinus setosus:																		
1,239	Developing series	50	4 to 15	100	50	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
		103	15 20	78	80	4	4	5	5	2	2	7	7	.....	.....	.....	.....	5	5
		270	20 25	51	138	1	4	16	43	11	30	17	46	0.4	1	.....	.....	3	8
		240	25 30	21	51	3	6	8	19	14	33	45	109	1	3	1	2	7	17
		245	30 35	10	25	2	4	12	29	5	13	63	154	4	10	.....	.....	4	10
		169	35 40	5	8	2	3	3	5	5	9	71	120	6	11	2	4	5	9
		162	40 50	4	6	1	2	0.6	1	6	10	70	113	9	15	6	10	3	5
				.....	358	.....	23	.....	102	.....	97	.....	549	.....	40	.....	16	.....	54
162	Developed series	162	50 80	.....	.....	.....	.....	0.6	1	0.6	1	73	118	13	21	10	16	3	5
1,401																			
400	*Toxopneustes variegatus*	.....	.....	.....	.....	0.5	2	0.3	1	84	335	15	58	.....	.....	.....	.....	1	4
	Tripneustes esculentus:																		
167	Developing series	10	20 25	.....	.....	50	5	.....	.....	50	5	.....	.....	.....	.....	.....	.....	.....	.....
		21	25 30	.....	.....	24	5	.....	.....	76	16	.....	.....	.....	.....	.....	.....	.....	.....
		60	30 40	.....	.....	12	7	.....	.....	80	48	2	1	2	1	.....	.....	5	3
		42	40 50	.....	.....	.....	.....	.....	.....	90	38	2	1	2	1	.....	.....	5	2
		34	50 70	.....	.....	.....	.....	.....	.....	53	18	18	6	6	2	.....	.....	24	8
				.....	.....	.....	17	.....	.....	.....	125	.....	8	.....	4	.....	.....	.....	13
407	Developed series	125	70 90	.....	.....	0.8	1	.....	.....	30	37	34	42	28	35	.....	.....	8	10
		200	90 110	.....	.....	0.5	1	0.5	1	22	43	24	47	40	79	2	4	13	25
		82	110 132	.....	.....	.....	.....	.....	.....	13	11	24	20	43	35	5	4	15	12
574				.....	.....	.....	2	.....	1	.....	91	.....	109	.....	149	.....	8	.....	47
	Echinometra lucunter:		Length.																
225	Developing series	34	4.5 to 15	100	34	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
		23	15 20	91	21	.....	.....	9	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
		58	20 25	71	41	.....	.....	29	17	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
		110	25 30	34	37	2	2	54	60	10	11	.....	.....	.....	.....	.....	.....	.....	.....
				.....	133	.....	2	.....	79	.....	11	.....	.....	.....	.....	.....	.....	.....	.....
278	Developed series	175	30 40	17	30	.....	.....	58	102	25	43	.....	.....	.....	.....	.....	.....	.....	
		103	40 52	7	7	.....	.....	66	68	26	27	.....	.....	.....	.....	.....	.....	1	1
503				.....	37	.....	.....	.....	170	.....	70	.....	.....	.....	.....	.....	.....	.....	1

the dental capsules are large, embracing the base of the tooth as in the Echinidæ and Strongylocentrotidæ. The perignathic girdle of *Echinometra lucunter* in the adult is highly specialized, having relatively high ridge-like apophyses and extravagantly developed auricles which meet over the ambulacra and are produced dorsally as spoon-shaped plates with a long median symphysis (fig. 21). In young specimens, 5 mm. in length, the auricles exist as simple erect styles situated on either side of the ambulacra but not arched over the same (fig. 18). In specimens 10 mm. in length, the auricles arch over the ambulacra, but are still separate, not meeting in median suture (fig. 19). In a specimen 15 mm. in length, the auricles have met over the ambulacra and are joined by median suture, and the apophyses exist as moderately developed ridges (fig. 20). From here on to the adult condition the apophyses increase in height and the auricles gradually take on an extensive vertical enlargement of their upper border (fig. 21). In *Echinometra viridis* A. Agassiz, *E. oblonga* Blainville, and *E. vanbrunti* A. Agassiz, in adults, the auricles are relatively slender, but are united in suture over the ambulacra; there is no vertical extension in a spoon-like fashion, however, and the auricles closely resemble the condition of *E. lucunter* when about 15 mm. in length.

In *Echinometra mathei* Blainville, in a young specimen 8 mm. long from Mauritius, the auricles are still separate, but in the adult the auricles are joined in suture and with moderately developed spoon-like processes, but not as high processes as are those in adult *E. lucunter*. In *Heterocentrotus trigonarius* (Lamarck), from Mauritius, a young individual 9 mm. in length, has the auricles still separate, but in the adult of this species auricles are arched and united over the ambulacra and with moderately developed spoon-like processes.

SUMMARY AND CONCLUSIONS.

Montego Bay, Jamaica, is an excellent locality for studying Echini on account of the number of species there available and the abundance of material. It is an ideal place for a student who wishes to supplement studies on northern species by observations on tropical forms.

Some 2,878 specimens of regular Echini were collected and studied, but in no case did any specimen show a departure from the typical pentamerous system. This is mentioned as in previous studies it was found that, on the average, completely or partially trimerous, tetramerous, or hexamerous specimens occurred a little oftener than once in a thousand specimens.

In the "Phylogeny of the Echini," page 91, it is stated that "all the evidence goes to show that the full number of oculars that are to become insert are developed early in the life of the individual and apparently later no change in this respect takes place." It was assumed that in Echini specimens of about half the mature size could be accepted as showing the mature characters as regards oculars, and the tables of ocular plate variation, while based principally on adults, were made up on the basis of considering specimens more than half-grown as being fully developed in this respect. This view was based largely on studies of *Strongylocentrotus dröbachiensis*, and in this species the view was fully justified. It is doubtless true of many species, perhaps most species of Echini, that specimens half-grown have practically the full character as regards ocular development. It is not entirely true, however, of all species, for as shown in this paper, in *Centrechinus setosus*, *Tripneustes esculentus*, and *Echinometra lucunter* (table 2), ocular plates continue to come in or enter the periproct as a developing character until nearly or quite grown; therefore, as a result in these species, the largest numbers in percentage of oculars insert may be found in the series of largest specimens. This is not true of selected individuals. That is, the largest specimen does not necessarily have more oculars insert than a smaller individual, but taking a large series of individuals of these species, as shown in the table, the average of each size up to the largest has more oculars insert than any of the preceding sizes. This late coming in of oculars in some species is of interest as showing the mobility in adjustment of the test to allow these changes to take place. It is also of interest in support of the view urged so much by Professor Hyatt, that changes are taking place constantly throughout the life of the individual.

Centrechinus setosus is slow and very gradual in developing its characters. It is slow in acquiring genital pores and very slow relatively in having ocular plates enter the periproct. Up to 15 mm. in diameter no oculars have yet entered the periproct. In the series 20 to 25 mm. in diameter, a little more than half of the specimens still have all oculars exsert. When 35 to 40 mm. in diameter, which is about half-grown for the locality, the species character of I, V, IV insert is developed to a high point, 71 per cent, practically the maximum percentage; but at this age there are more specimens representing developing characters and fewer representing progressive characters than in older series. The maximum of the species character and also the maximum of the progressive characters of I, V, IV, II, and all oculars insert, is seen in the largest specimens of 50 to 80 mm. diameter. The slow rate of development of *Centrechinus* as here shown (p. 156) is graphically brought out by comparing it with that of the development of *Strongylocentrotus dröbachiensis* given in the "Phylogeny of the Echini," page 142. *Centrechinus* is a relatively primitive (and geologically old) genus in the lowest suborder (the Aulodonta) of the Centrechinoida. On the other hand, *S. dröbachiensis* is a highly accelerated species in the highest suborder (the Camarodonta) of the Centrechinoida. As Professor Hyatt showed fully in his studies of fossil Cephalopoda, primitive genera have a slow development and specialized genera have a highly accelerated development. I have shown the same thing in Pelecypoda where the primitive *Avicula* and *Pecten* have a relatively slow development, whereas the specialized *Spondylus* and *Ostrea* have an accelerated development.

Another feature of note in the ocular arrangement of *Centrechinus setosus* is the fact that while ocular I or ocular V may be the first to enter the periproct, V is by far the more common, and in so far makes an approach to the character of the Cidaridæ where ocular V alone is frequently insert, but I alone is rarely insert. In higher groups of the Centrechinoida, when one ocular is insert, the order is more definitely fixed, as in the Arbaciidæ and Echinometridæ, where V is typically and I is rarely insert; or in the Echinidæ and Strongylocentrotidæ, where I is typically and V is rarely insert. Another ocular peculiarity of *Centrechinus* is that where four plates reach the periproct, I, V, IV, III insert is a rather frequent aberrant variant. This is a very rare character in the higher Centrechinoida but is a common character in the Cidaridæ.

Of the ocular characters of *Toxopneustes variegatus*, no special comment is called for. It is simply the record from a definite locality, whereas my previous record of the species was based on specimens from several localities. The peculiar zigzag pattern of color ornamentation shown recalls the somewhat similar pattern seen in some of the Temnopleuridæ.

In *Tripneustes esculentus* the ocular arrangement (figs. 10–17) is one of the most interesting that has been found in any Echini. Two oculars insert is commonly given as the species character, and it is the dominant character in some localities (Bermuda, Florida). In Montego Bay this character is acquired up to 90 per cent in specimens 40 to 50 mm. in diameter, much less than half-grown. But in Montego Bay, as a local character, the species has gone much further and passed through a phase in which oculars I, V, IV are insert like Bahama material, and finally takes on oculars I, V, IV, II insert as the fully matured adult character. It is an exceptionally interesting case as being the acquirement in late stages of growth as a typical feature of a character which is a normal progressive variant of its own species in other localities and also a typical advanced character in all Echini which have four oculars insert. Looking at the test of a nearly grown *Tripneustes* with its solid heavy plates, it is astonishing to realize that they are still plastic and capable of allowing the readjustment of parts indicated by the separation of genitals and traveling in of oculars so as to reach the periproct. Not less surprising is the power of the law of ocular development, that not any ocular, but a certain specific ocular, is the one that is selected by the laws of growth to change its relative position. I know of no more striking case of the definiteness of action in the growth of organisms.

As stated, *Echinometra lucunter* is one of the very few species of Echini characterized by having one ocular insert; therefore a series showing its development is of interest. Four of the six species in the genus *Echinometra* are characterized by having all oculars exsert as the species feature, and in these to have one ocular insert is a progressive variant. Also as a family character, all exsert is the leading feature. Therefore for the genus and for the family, *Echinometra lucunter* can be considered an advanced type as regards ocular arrangement. In accordance with this fact it is not strange, it might even be expected, that it would take on its advanced character of oculars insert at a somewhat late stage as it does. The dominance of V insert, 54 per cent, is first attained when the specimens are 25 to 30 mm. in diameter, about half grown, and the relative increase of V insert progresses somewhat up to the largest series recorded. Having oculars V, I insert is a progressive character for this species, and this feature comes in late, progressively increasing in frequency up to the largest size recorded. To have oculars V, I insert as a typical character is the feature of *Echinometra van brunti* A. Agassiz, from Lower California and South America, and in this respect it is the most specialized species in the genus and family.

In the "Phylogeny of the Echini" much attention was given to ocular plate arrangement and development of the same. The results for the Centrechinoida are there briefly summarized in pages 92–94. As observations on the Montego Bay series have materially extended the results in some important particulars, a brief statement of the laws of ocular arrangement is given here. In the "Phylogeny" the results of tabulating ocular plates in young and adults of the order Centrechinoida are given for 48,541 specimens. Those given above include in addition 2,878 specimens, or including both, 51,419 specimens. By the law of ocular development as worked out in this order, the oculars when entering the periproct, do so in the sequence I, V or V, I, IV, II, III (figs. 10–14), and it is seen by the following how closely this rule is adhered to. Of the total 51,419 specimens of Centrechinoida observed, oculars are all exsert in 6,763 specimens, all others having one or more oculars insert. In 3,708 specimens, one ocular is insert, and of these in 3,679 cases it is either ocular I (fig. 10) or ocular V, that is, 99.22 per cent are correct by rule. There are only 29 exceptions.[1] In 35,951 specimens two oculars are insert, and of these, in 35,611 cases, it is oculars I and V, or the bivium (fig. 11), that is, 99.05 per cent are correct by rule. In all of the 340 exceptions[2] one of the two plates insert is either I or V. In 3,820 specimens, three oculars reach the periproct, and of these, in 3,426 cases, it is oculars I, V, IV, the bivium and the left posterior plate of the trivium (fig. 12), that is, 89.68 per cent are correct by rule. Of the 394 exceptions in 360 cases, the order is I, V, II insert (fig. 15), that is, the right posterior plate of the trivium is insert instead of the left posterior as usual. These may therefore be considered as right-handed specimens. Of the total 3,820 specimens with three oculars insert, 3,786, or 99.37 per cent, have either oculars I, V, IV, or oculars I, V, II insert.[3] In 619 specimens, four oculars reach the periproct, and of these in 554 cases, it is oculars I, V, IV, II the bivium and posterior pair of the trivium (fig. 13); that is, 89.50 per cent are correct by rule.[4] In 558 cases all five oculars are insert (fig. 14). In the

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[1] Of these exceptions, 18 have ocular IV alone insert, three have ocular III alone insert, and eight have ocular II alone insert.

[2] Of these exceptions, in 101 cases, oculars I, IV are insert; in one case oculars I, III are insert; in 56 cases oculars I, II are insert, which is the species character in *Gymnechinus robillardi* (Loriol) and *G. pulchellus* Mortensen; in 113 cases oculars V, IV are insert; in 4 cases oculars V, III are insert; and in 65 cases oculars V, II are insert.

[3] Of the other 34 exceptions to the rule where 3 oculars are insert, in 4 cases oculars I, V, III are insert; in one case oculars I, IV, II are insert; in 11 cases oculars V, IV, III are insert; and in 18 cases oculars V, IV, II are insert.

[4] Of the 65 exceptions in 64 cases, the arrangement is oculars I, V, IV, III insert (fig. 16); this character which is rare in the Centrechinoida as a whole, occurred principally in *Centrechinus setosus* (47 cases), but it is a common character in the Cidaroida, the other 17 cases of I, V, IV, III insert are scattered through a number of species of the Centrechinoida. One aberrant with four oculars insert has oculars I, V, II, III reaching the periproct (fig. 17). This, which may be considered a right-handed equivalent of I, V, IV, III as far as experience goes, is a unique variant in Echini.

"Phylogeny" a detailed discussion is given of the bearing of aberrant variants which, although aberrant, follow quite definite lines of their own. Of the total 51,419 specimens considered above, 50,591, or 98.39 per cent, are correct by rule as regards ocular arrangement, and 828 specimens (of which 360 are I, V, II as above noted), or 1.61 per cent, have an aberrant arrangement.

From the above records it is seen that ocular plates in the Centrechinoida follow a very definite line as regards which ones reach the periproct, and by their arrangement emphasize a bilateral symmetry through the axis of ambulacrum and ocular III and interambulacrum and genital 5. This is the axis on which the irregular types develop an elongate form, eccentric periproct and other features of bilaterality, and it was urged by Lovén as the correct axis for orienting Echini. Mr. A. Agassiz (*Challenger* Echini, pp. 4–6) criticizes Lovén's determination of the antero-posterior axis in Echini. His argument, however, simply shows that he misunderstood Lovén's point of view.

In accordance with what I previously showed in *Strongylocentrotus* it is seen that the perforation of the genital plates by the genital pores originates at quite definite periods. In *Centrechinus setosus* the genitals are imperforate in specimens up to 14 mm. in diameter, after which with few exceptions all specimens have developed the genital pores. In *Echinometra*, genitals are imperforate up to 10 mm., after which all plates are perforate. Both of these are later in developing than *Strongylocentrotus dröbachiensis*, which is imperforate only up to 5 mm. in diameter, after which in most cases the genitals are perforate.

In *Eucidaris tribuloides* and *Centrechinus setosus* the teeth extend to the upper line of the lantern, but do not extend horizontally over the top of the same; also, in both the dental capsules embracing the base of the teeth are small. These are both doubtless primitive conditions, and are opposed to the condition found in the Echinidæ and Strongylocentrotidæ. In *Toxopneustes variegatus*, *Tripneustes esculentus*, and *Echinometra lucunter* the teeth extend horizontally over the top of the lantern, the proximal end of the tooth is grooved, not keeled, the keel appearing further down the tooth, all as in *Strongylocentrotus*. This grooved character is of interest as a localized stage in development, as it shows that the young growing point of the tooth has the character retained throughout life in the Aulodonta, Cidaroida, and Palæozoic genera, in which the teeth are always grooved. In *Toxopneustes*, *Tripneustes*, and *Echinometra* the dental capsules are large, here again being like the character of *Strongylocentrotus* and differing from the condition in *Eucidaris* and *Centrechinus*.

In *Centrechinus setosus* the perignathic girdle is specialized in the adult, with high apophyses and high laminar auricles joined over the ambulacra. In the young, the apophyses are very slight and the auricles are simple spur-like processes, as in the adult Aspidodiadematidæ, which represent the simplest auricles known. Later, in *Centrechinus*, the auricles arch over the ambulacra, but without fusion. Then they fuse in delicate arches, a condition comparable to that of the adults of *Chætodiadema pallidum* A. Agassiz and Clark and *Centrostephanus rodgersi* A. Agassiz. Later still, in *Centrechinus*, the auricles develop laminar expansions on their upper border, which in large specimens become extensive.

In *Echinometra lucunter*, the perignathic girdle is also very specialized, attaining an extravagant development of the auricles. In the young, however, the auricles exist as simple separate styles. Later they arch over the ambulacra, but without being in contact; still later they join over the ambulacra as delicate arches, a condition comparable to that seen as the adult character in simpler species of the genus. Finally, the auricles take on a great development of the dorsal border, producing the spoon-like arches characteristic of the species.

As shown and urged by Hyatt from his studies of Cephalopoda, and as I have shown in previous studies, in the present study of Echini, and in plants, development, that is the addition of differential characters, continues more or less markedly throughout the life of the individual, and a study of such development yields facts of value in the structure, the morphology, and the phylogenetic relations of the group in hand.

X.

THE SPERMATOGENESIS OF THE MONGOOSE; AND A FURTHER COMPARATIVE STUDY OF MAMMALIAN SPERMATOGENESIS, WITH SPECIAL REFERENCE TO SEX CHROMOSOMES.

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One plate and nine text-figures.

THE SPERMATOGENESIS OF THE MONGOOSE; AND A FURTHER COMPARATIVE STUDY OF MAMMALIAN SPERMATOGENESIS, WITH SPECIAL REFERENCE TO SEX CHROMOSOMES.

By H. E. Jordan.

INTRODUCTORY.

In view of the recent discovery of heterochromosomes in a number of the higher vertebrates it seemed desirable that the spermatogenesis of still other forms should be carefully studied with particular attention to the possible presence and variable behavior of homologous "accessory" chromosomal elements. While this study was in process opportunity was afforded to collect in Jamaica, British West Indies, material of the mongoose.[1] This material is interesting in a number of respects and serves well as a starting-point for such comparative study as will appear from its description. Other material at hand includes that of cat, squirrel, pig, rabbit, white mouse, sheep, horse, mule, bull, and dog.

HETEROCHROMOSOMES LACKING IN MALE.

MONGOOSE.

Spermatogonial nuclei more generally contain three plasmosomes (fig. 1), though there may be more or fewer. Only a very few nuclei were seen in mitosis. Many stages of apparent amitosis are present (fig. 2). The rarity of mitotic figures and of binucleate cells indicates that the prevailing type of division among the spermatogonia is amitotic. The presence, however, of a few binucleate spermatocytes and spermatids shows that cell-body divisions do not always follow the nuclear divisions.

The primary spermatocytes contain a single nucleolus (plasmosome), seen only in the resting condition of the nucleus. The fact that this body never appears subsequently makes the synapsis and division stages of the mongoose especially favorable for study with reference to the presence of an "accessory chromosome." There can be no confusion here between a plasmosome and a chromosome-nucleolus, as has apparently been the case in some forms. Moreover, the early disappearance of the plasmosome in mongoose gives support to the general conclusion that synaptic and post-synaptic chromatin elements are chromosomes, more especially when invariably on the nuclear membrane instead of being disposed at random.

The synizesis phase is characterized by a very compact, deeply chromatic mass of simple threads (fig. 4). The mass is situated at the pole of the nucleus, frequently, but not invariably, next the idiosome. The remainder of the nuclear space remains empty, except for a few delicate linin threads, more abundant in the vicinity of the mass. All about the idiosome are pale spherical mitochondria, now apparently present for the first time; but no clue appears as to their origin.

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[1] This opportunity occurred during a month's stay at the temporary Marine Biological Laboratory of the Carnegie Institution of Washington at Montego Bay in the spring of 1912. I take this occasion to express my thanks for the privileges of the Institution's expedition to Jamaica, and for daily kindnesses on the part of the director, Dr. Alfred G. Mayer.

The fact that the synizesis nucleus is not appreciably larger than that of the resting stage shows that synizesis is not due to mechanical factors consequent upon a great increase in nuclear sap. On the contrary, this phenomenon is unquestionably the result of the specific activity of the chromatic nuclear thread. Its frequently polar position may be due to idiosome influence.

In the bouquet stage the simple threads emerge from the synizesis mass in pairs (fig. 5) and unite side by side (parasynapsis). The nucleus is still of approximately the same size; and again the chromatin threads give evidence of a specific activity. The process continues until the previously empty nucleus is filled with double threads crossing in every direction throughout the nucleus (fig. 6). Subsequently the nucleus enlarges somewhat, and the double threads shorten and thicken (fig. 7). The beginning of division of the idiosome marks this as the early prophase. The bivalent chromosomes appear more chromatic and knobbed at the ends, an early indication of tetrad formation. Figure 8 illustrates a later prophase stage, in which the chromosomes have become still shorter, uniformly chromatic, and more compact, a number appearing as complete tetrads.

The point of special interest is that nowhere throughout these early phases has the slightest evidence of an accessory chromosome appeared. No evidence would be expected to appear subsequently, and this is actually the case, as shown in figures 9 to 17. At metaphase the chromosomes are all arranged in a very compact equatorial plate (figs. 9 and 10). No chromosome seems marked by peculiar behavior or uncommon size. An accurate count seems impossible. Innumerable division figures appear, but the chromosomes are so closely compacted as to preclude definition of limits. A most careful study warrants only the statement that the number of chromosomes is somewhere around 24.

A resting stage ensues, but without chromosome-nucleolus, in contradistinction to the usual condition in forms with an accessory chromosome, or without even a plasmosome. The division figures of the second mitosis are smaller, somewhat less compact, and seem to consist of fewer chromosomes. We may be dealing here, as in the opossum, with a second pairing, or a hemioid group (Jordan, 1911). The smaller size of the chromosomes, however, makes this a little doubtful.

Figure 14 illustrates a resting spermatid, with conspicuous idiosome. At the next step in the metamorphosis (fig. 15) the cell has elongated, the nucleus has become more dense and in consequence smaller, and the idiosome has given place to the archoplasmic sphere. The latter contains centrally a chromatic granule, presumably the centrosome (fig. 15). The sphere seems to have arisen directly from the idiosome by process of vesiculation and rejection of one or several small plastin particles. The condensing nucleus moves into an elongating projection of the cell, and then to and beyond the periphery (figs. 16 and 17). Meanwhile the "sphere" has flowed backward over the elongating nucleus, its lateral limits being marked by a distinct line for some distance on either side of the growing axial filament. The filament is marked proximally by two granules (the products of the original centrosome, fig. 15), the anterior one at the point of attachment to the nucleus, the posterior one passing along the axial filament as the "ring centrosome" (figs. 17 and 18) to the posterior end of the middle piece. At the later and adult stages the middle piece is attached to the head by a delicate neck (fig. 20, drawn in the living condition), in which appears a knob, the proximal centrosome, or "end knob" (figs. 19 to 21). The posterior limit of the middle piece is defined by the termination of the spiral filament. The latter arises by a coalescence of mitochondria included within the limits of the backward-extending sphere (middle piece). The remaining mitochondria fail of inclusion and are cast off with the discarded cytoplasm. This observation, made also in a number of other forms (*e. g.*, opossum, Jordan, 1911; *Euchistus*, Montgomery, 1912), invalidates any hypothesis attributing specific hereditary significance to mitochondria.

CAT.

The later steps in the spermatogenesis of the cat, including the spermatid and subsequent stages, have recently been described by Leplat (1910). As to the prespermatid stages, it may be said in brief that they are essentially similar to those described for mongoose, and no convincing evidence appears at any stage of heterochromosomes. Respecting the postspermatid stages also, the similarity amounts practically to an identity; and my own observations on these stages agree essentially with those made and illustrated by Leplat.

More recently Gutherz (1912) has described and illustrated (in two figures) first spermatocytes of the cat stained both in the iron-hematoxylin and the Biondi mixtures. His conclusion confirms my own as to the probable absence of heterochromosomes. However, he figures a synaptic (synizesis) and a postsynaptic stage in which appear heterochromosome-like elements in iron-hematoxylin preparations. The true acidophile nature of these bodies, however, is revealed in the Biondi material. On the basis of this observation he casts doubt upon the interpretation as a heterochromosome of the element described by Winiwarter and Sainmont in their iron-hematoxylin preparations of the oöcyte of the cat. I can only add that my material (also stained with iron-hematoxylin) reveals elements somewhat similar to those described and illustrated by Gutherz for the first spermatocytes, but which, on grounds of morphology, location, relation to spireme, and general behavior, I can not interpret as typical X-elements. Moreover, in well-decolorized preparations their non-chromatic nature is likewise revealed. The illustrations of the oöcytes of the cat by Winiwarter and Sainmont (see figs. 27*a*, 33, and 44) answer to the main criterion, namely morphological, for the presence of a heterochromosome, "split monosome." A comparison of the illustrations of the spermatocytes and oöcytes of the cat as given by Gutherz and by Winiwarter and Sainmont respectively does not seem to warrant the conclusion of an homology between the hypothetical X-element in the male and that in female germ-cells of the cat. In short, the oöcytes would seem to contain, the spermatocytes to lack, typical X-elements.

SQUIRREL.

The spermatogenesis of the squirrel has been described in two papers by Van Mollé (1906, 1907). The cells are relatively very large and the idiosome is very distinct. My observations agree with the illustrations of Van Mollé with respect to the absence of heterochromosomes. Duesberg (1910) has recently questioned the accuracy of Van Mollé's observations with respect to the details of the later stages in the metamorphosis of the spermatid. Interest in these details is outside the scope of this contribution, and my observations on these points have not yet been carried to an extent where I might confidently presume to express an opinion. The absence of any accessory chromosomal element during the growth period appears unquestionable.

PIG.

It will suffice for present purposes to state simply that evidence of an accessory or heterotropic chromosome at early stages of the spermatogenesis of the pig is completely lacking in my material. The cells here are relatively small; but the fixation is good and reveals clearly all the details of structure, and every phase is abundantly represented prior to the division phases; occasionally first maturation groups are also present.[1]

RABBIT.

Exactly the same statement holds for the rabbit as for the pig; and I make it with greater confidence, since the material is more extensive including besides adult testes those of new-born and young individuals.

In all of the foregoing instances the spermatogonia contain plasmosomes. The resting primary spermatocytes also contain either plasmosomes or karyosomes (net-knots), or both, all of which disappear in early presynapsis. It seems legitimate to assume, on the basis mainly of this observation, that in mammals generally plasmosomes almost invariably disappear before synapsis, and can thus produce no confusion at those stages where heterochromosomes when present are most conspicuous, *i. e.*, synaptic and early postsynaptic stages. The conclusion that heterochromosomes are wanting in the male of the mongoose, cat, squirrel, pig, and rabbit is, of course, based on the assumption that if present they would be conspicuous at the same stages in which they are so strikingly in evidence in the group of mammals next to be described. The final test must, however, in these cases be an actual count of chromosomes.

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[1] However, no chromosome counts were attempted. In view of J. E. Wodsedalek's recent work ("Accessory Chromosomes in Pig;" Science, N. S., vol. XXXVIII, No. 966, pp. 30–31) reporting two additional chromosomes or accessories in half of the secondary spermatocytes—this number (10) corresponding with the reduced number in the eggs—the pig can no longer be listed under "exceptions." In the absence of definite evidence during the growth period, the final criterion for the presence of heterochromosomes must be an actual count.

Since the appearance of Wodsedalek's complete paper (Biol. Bull., 25: 1), I have carefully re-examined my sections of the pigs' testes. Mitoses are too infrequent to warrant any statement concerning the presence or absence of accessory chromosomes on the basis of appearances at metaphase, or of chromosome counts. My conclusions rested upon evidence drawn from a study of appearances during the growth stages including the early prophase. As to the resting spermatogonial and primary spermatocyte nuclei I find no such constancy with reference to nucleoli as Wodsedalek records. The number, both of the large and smaller nucleoli, is very variable. Three or four large nucleoli are common. Moreover, with reference to the chromosome-nucleoli I can not find in my preparations (of young testes) any cells to parallel his illustrations 21, 23, 24, and 25. My material does not confirm his finding that "two large round nucleoli remain very conspicuous throughout the process of growth of the primary spermatocyte."

HETEROCHROMOSOMES PRESENT IN MALE.

WHITE MOUSE.

Spermatogonial and spermatocyte resting nuclei agree in being vesicular and having a delicate chromatic reticulum with numerous net-knots (figs. 22 and 23); they differ in that the spermatocyte nucleus has a bilobed or double chromatin (chromosome) nucleolus.

The Sertoli cell has a still more vesicular nucleus, which is very conspicuous by reason of a characteristic trilobed, deep-staining nucleolus, the central section being invariably the larger. This peculiar type of nucleolus is as characteristic of the Sertoli cell as its peculiar shape, and suggests interesting speculations concerning the relationship between nucleolar morphology and cell functions.

My earlier observations led me to the conclusion that the synapsis phenomenon (*i. e.*, synaptic knot, contraction or bouquet phase, or synizesis stage)[1] was lacking in the auxocytes of the white mouse. Further careful search has disclosed a few cells in the condition illustrated in figure 24. This is tentatively interpreted as synapsis (polarized amphitene). If the nucleus is normal, as it appears to be, the paired threads unmistakably indicate synapsis. I find that Regaud (1909, 1910) reports his failure to find a synapsis phase also in the rat. This phase is assuredly not accentuated in white mouse. Nothing more closely resembling synapsis than the nuclear arrangement illustrated in figure 24 could be found in my material. This, however, seems sufficiently suggestive of true synapsis to show that something similar to synapsis actually occurs in the mouse, perhaps too rapidly to appear except very occasionally in "fixed" preparations. If the phenomenon is really lacking the "synapsis" figure does not appear essential to the pairing of the chromosomes in the formation of the haploid group.

During synapsis (parasynapsis) and early postsynapsis (figs. 24 and 25) the chromatic accessory body (chromosome) is apparently usually single, usually oval, sometimes slightly bilobed, and occasionally wedge-shaped or even irregular. During later postsynaptic (fig. 26) and prophase (fig. 27) stages, this element again appears invariably double, frequently in the form of a longitudinally split rod. There is no unequivocal evidence that the pair may entirely and widely separate, as is the case in the mule for example. The double nature of the heterochromosome suggests a pair of idiochromosomes; but the fact that during and immediately after synapsis this element appears single invalidates somewhat such an assumption, and suggests rather a double accessory chromosome, or X-element of Wilson. In leptotene and diplotene phases it very frequently lies in a clear space free of threads, a condition similar to that described by Wilson (1912) for *Largus* and *Oncopeltus*.

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[1] Synapsis is assumed to take place during the period of contraction or "synizesis"; hence the terms are here used interchangeably.

Figure 27*a* would seem to permit of not the slightest doubt of the presence of a "sex chromosome" (Wilson) in the white mouse. Its later history, however, is still obscure. Division figures are very abundant in my material and the preservation is nothing short of superb, but at no later stage can I with certainty identify this body. Occasionally a spindle appears with a large chromosome in advance of the main metaphase group (fig. 27*b*), but the instances seem too rare to have much significance apart from morphological marks of identification from among the chromosomes of the metaphase plates.

SHEEP.

In sheep I am able to distinguish the "X-element" during the presynaptic (fig. 28), synaptic (fig. 29), and postsynaptic (fig. 30) stages. Both in the first and second stages it is usually attached to the chromatic spireme, or one of its segments. During the postsynaptic stages it is usually split or bilobed, and, as at earlier stages, frequently but not invariably lies close to the idiosome. Synapsis is effected by side-by-side union. In the sheep, also, this accessory chromosome eludes later certain identification.

HORSE.

As previously briefly noted (Jordan, 1911, 1912), during postsynaptic stages the horse shows frequently a tripartite X-element (fig. 33), usually next the idiosome. In the resting phase of the primary spermatocyte (fig. 31), and during synapsis, it appears double. Further clear tracing of this body seems impossible. The evidence is thus far from sufficient to warrant even an inference as to whether we are here dealing with a multiple X-element (as in *Sinea*, for example, according to Payne, 1909) or a pair of idiochromosomes. Both conditions have been described for mammals; for example, man as having a double X-element (as in *Syromastes*—Wilson, 1909) by Guyer (1910), and guinea-pig with a pair of heterochromosomes, by Miss Stevens (1912). The only point sought to establish is the presence of "sex chromosomes" of some sort.

Kirillow (1912) has recently published the first number of his projected studies on the spermatogenesis of the horse. The accompanying illustrations, including all the chief stages, give no indication of a heterotropic body so conspicuous in my preparations; nor is any mention made of such body. His observations on the germ-cells of the horse lead him to accept the opinion of Regaud (1909, 1910) that "the appearance or failure of synapsis is a variable factor in different species" (Kirillow, p. 145); that is, synapsis (contraction phase) is regarded as a normal phase in the auxocytes of some forms (species), but is thought not to appear in others.

MULE.

The presence of an accessory chromosome in the mule was also noted briefly in an earlier paper (Jordan, 1911, 1912). Here it usually appears double in pre- and post-synaptic stages (figs. 34 and 36). At synapsis it appears as a single large oval chromatic body (fig. 35). It is very clearly recognizable among the prophase chromosomes as a large paired element (fig. 37). Since at certain phases (synapsis) it may be a single compact body, it is perhaps more legitimate to regard it as a double X-element.

In the case of the mule it will be impossible further to trace this body with any degree of certainty. As first noted by myself (see quotation from my unpublished manuscript in a paper by Dr. R. H. Whitehead, "A Peculiar Case of Cryporchism, and its Bearing upon the Problem of the Function of the Interstitial Cell of the Testis," Anat. Record, Aug. 1908; vol. II, No. 5), spermatogenesis in the mule does not ordinarily proceed beyond the early prophase. This is the cause of the sterility of mules bred *inter se;* the mule does not generally produce ripe spermatozoa. My observations were subsequently confirmed by Poll (1911), who succeeded in observing a few first maturation spindles at metaphase.

BULL.

The spermatogenesis of the bull has been fully described by Schoenfeld (1901). My observations agree essentially with his illustrations. Schoenfeld figures a stage similar to my illustration of postsynapsis (fig. 38), where a single large, oval, chromatic "accessory chromosome" appears among the bivalent threads, close to the idiosome. This element, at this as well as at earlier and later stages, Schoenfeld describes as the "corpuscle intranucleare." In the light of what we now know of "sex-chromosomes" generally, and in mammals more particularly, this body may, I believe, be regarded confidently as an accessory chromosome.

DOG.

Figure 39 illustrates a resting primary spermatocyte with pale plasmosome and chromatic, bilobed chromosome nucleolus. No very satisfactory synapsis stages could be observed in my material.[1] Figures 40 and 41 illustrate two successive postsynaptic phases in which the X-element is conspicuously present as a chromatic, sharply contoured, irregularly oval body close to the idiosome. We are probably here again dealing with an accessory chromosome. The attempt further to trace this body, however, was very disconcerting by reason of the absence of definite marks of identity, lack of clear delimitation of the relatively large number of small chromosomes, and confusing contradictions of trying observations. In short, any further analysis was unsuccessful.

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[1] That is, from the standpoint of a conspicuous "X-element."

DISCUSSION.

Heterochromosomes are apparently lacking in mongoose, cat, squirrel, pig, and rabbit, and appear to be present in white mouse, sheep, horse, mule, bull, and dog. On the basis of what was already known regarding man (Guyer, 1910; Gutherz, 1912),[1] rat (Guyer, 1910), armadillo (Newman and Patterson, 1910), guinea-pig (Stevens, 1912), and opossum and bat (Jordan, 1911, 1912), heterochromosomes were suspected in mammals generally. With respect to heterochromosomes, then, mammals fall into two groups: those lacking and those possessing the X-element in the male germ-cells. Under the first group five[2] mammals can be classified; under the second, twelve. Both groups include higher as well as lower mammals. Measured by no other criterion known to me can these mammals be similarly grouped. Presence of heterochromosomes obviously bears no relationship to natural affinities or evolutionary levels of mammals. The proportion of 12 to 5 among examined and recorded cases of mammals in favor of those possessing heterochromosomes suggests that in those forms apparently lacking them they have simply eluded detection. They might presumably be so small as to escape recognition, or lack the peculiar morphological marks by which they are usually characterized, or be too labile to be identified.

Regarding a similar condition in *Culex*, Stevens (1911, 1912) suggests that here, "where no heterochromosome differentiation of any kind has been detected, the members of a pair of chromosomes may differ by a sex unit or some other character unit, and the difference in size comes within the probable error of most careful observation" (p. 165).

The observation of the attachment of the accessory chromosome to the parasynaptic spireme (figs. 28, 29, and 40) controverts the plausibility of Stevens's (1912) suggestion that "the condensed condition of the odd chromosome and the unequally paired heterochromosomes of the growth stage of the first spermatocytes might be due to their unpaired or unequally paired condition preventing them from joining with the other bivalent chromosomes to form a spireme, especially in cases of parasynapsis" (p. 166).

It would seem to be required, then, for a proper grounding of later hypotheses, that all uncertainty be disposed of, first with respect to the possible presence in some form or at some stage of an X-element in those animals in which it seems to be wanting; and second, with respect to possible confusion with a persisting plasmosome in those instances where an X-element is believed to obtain. Though the above order would seem to be the more logical, the best approach to the argument and the proof is perhaps by way of the second point, though in fact they sustain a reciprocal relationship.

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[1] Gutherz (1912) confirms Guyer's (1910) observation respecting the presence of a heterotropic element in man, but interprets it as a pair of equal idiochromosomes undergoing neither heterokinesis nor giving rise to a dimorphism of spermatozoa as described by Guyer.

[2] This must be a tentative grouping; only four mammals can now be temporarily placed in this group.

Regarding a possible confusion between plasmosomes and accessory chromosomes, or X-elements, it must be noted first that in the stain employed (Heidenhain's iron-hematoxylin; checked in doubtful instances by Auerbach's methyl-green-acid-fuchsin stain) the plasmosome when present (as in spermatogonia and occasionally in primary spermatocytes) is always in properly decolorized preparations considerably less deeply stained than the X-element. Moreover, the latter is frequently attached to the spireme or one of its segments, and generally holds a position close to the nuclear wall and close to the point where the idiosome lies. Again, it is frequently bilobed or compound. In certain instances, *e. g.*, mouse more particularly, one of the first-division chromosomes occasionally passes to the pole in advance of the mass; but in no instance could the X-element be followed with any satisfaction or certainty beyond the early prophase. At this stage, however, it is in at least a number of instances clearly recognizable among the pale, mossy chromosomes as the body earlier identified as the X-element. The evidence would seem amply adequate to support the interpretation here as a typical X-element.

Heterotropic nuclear elements must be identified by other criteria than staining reaction. At best, staining capacity can only give confirmatory evidence. Morphological marks would seem to be the most certain grounds for basing distinctions. The complete nuclear history is of course necessary for full certainty. Chromosomes undergo alterations in their chromaticity and consequent staining capacity at different phases of the nuclear cycle. Again, true chromatin-nucleoli occur in certain forms (*e. g.*, oöcytes of *Asterias forbesii*, Jordan, 1908; oöcytes of *Echinaster* and *Cribrella*, Jordan, 1910), whose function at least in part is to contribute chromatic material to the chromosomes just before they enter the first maturation spindle. The plasmosomes of certain forms may thus be of true chromatic nature. Moreover, true nucleoli never assume—except perhaps very exceptionally and atypically—the characteristic bilobed and split forms of the chromosome-nucleoli during the growth stages. Degenerating nucleoli become ragged of outline, frequently fragment, and undergo karyolysis, but never show the series of phenomena characteristic of heterochromosomes: sharp contour, deep basophily, frequently split form, frequent attachment to spireme, and usual location close to the nuclear membrane. A presumptive chromosome-nucleolus may meet the test of basophilic staining reaction in a differential dye, but if it does not meet at least the majority of the above structural and spatial tests it very probably is simply a chromatic plasmosome; on the other hand, if it answer to the latter tests, confirmatory—but perhaps not crucial, in view of the undoubted chemical changes which chromosomes undergo—evidence accrues from a chromatic reaction to a "specific" stain.

Regarding the first stated point of possible doubt, then, nothing further can be said than that in the five forms enumerated as lacking an X-element, the tissue was similarly well preserved and stained, but notwithstanding careful search, not the slightest evidence of such an element in its usual morphological form and peculiar behavior with reference to spireme and idiosome could at any stage be detected; but this negative evidence obviously can not remove all uncertainty with respect to this first point.

Heterochromosomes (allosomes, Montgomery) in mongoose, and other forms showing like growth-stage conditions, may possibly have disappeared by close union with one of the ordinary chromosomes and thus forced into the usual behavior of the autosomes during auxocyte phases. Transition stages in such process of disappearance are apparently shown in certain orthoptera (McClung, 1905) and amphibia (*e. g.*, *Necturus*, King, 1912).

Militating further against the objection of mistaken identity is the fact that in this group the plasmosome has almost invariably disappeared[1] before synapsis, when the X-element first exhibits its characteristic behavior. If plasmosome could be confused with X-element in mammals, a group of five, with no relatively closer affinity than obtains among the contrasted group, could hardly be expected to give consistent and unequivocal evidence in favor of complete absence. Moreover, if the plasmosome generally disappears before or about the time of synapsis in a relatively no more closely related group of five forms, it seems quite legitimate to infer that it disappears at about the same time in six other—and as closely related—forms, and thus leaves little opportunity for confusion. And the above deduction is supported to some extent by direct observations (exception: man).

Having disposed of these objections as far as possible within the limitations of microchemical technic, it remains to consider further bearings and implications of the facts established. The observation of an X-element in the ovary of young cats (fig. 42) by Winiwarter and Sainmont (1909) has important significance in this connection. If my negative evidence for the male germ-cells in the cat can be regarded as equally certain with the positive evidence of these investigators for the female germ-cells, then it would seem that in those forms in which the males lack a typical X-element this is possessed by the female.[2] An analogous instance from the invertebrate group is that of certain sea-urchins reported by Baltzer (1909). Here the female possesses a pair of idiochromosomes, while the male lacks any indication of such. The above hypothesis for mammals must be tested by examination of the young ovaries of those forms in which the male appears to lack the accessory.

This hypothesis would be somewhat weakened if cases were known in which both male and female germ-cells possessed typical[3] X-elements. The meager evidence available is contrary to such a hypothetical condition. Wilson (1905, 1906) was unable to find a chromosome nucleolus in certain genera among the insects, including *Euchistus*, in which he was confirmed by Foote and Strobell (1909). Buchner's (1909) evidence with respect to *Gryllus*—the only presumptive contradictory evidence known to me—

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[1] A delayed disappearance occasionally occurs, more especially in cat and man.
[2] Presumably by approximately half of the ova.
[3] That is from the standpoint of behavior during the growth stages.

judged from his illustrations, seems far from conclusive. In fact, the hypothetical so-called X-element ("accessory body") in the ovary lacks much of being similar in structure and behavior to the undoubted X-element in *Œdipus* (described and figured in the same paper) and the male *Gryllus.* Moreover, Gutherz (1910) has shown that the two X-chromosomes, characteristic of the female, are both present in the metaphase plates of the oögonia, in addition to the outlying "accessory body." As is now established beyond any doubt the accessory, for example, of the male germ-cells passes into all females. Its homologue is present in both males and females; but the additional male accessory, at any rate, would seem to be able again to comport itself during the growth stage of the maturing oöcyte as it originally behaved in the growing spermatocyte; and similarly with respect to its female homologue, since that in the male, coming originally also from the egg, behaves again in typical "accessory" fashion. It seems fairly well established that the female homologues of the male accessory do not behave in the growing oöcytes as does the X-element in the spermatocyte.

As indicated by observation on the cat, the reverse of the usual relationship here obtains: the oöcytes are probably dimorphic with respect to an accessory; the spermatocytes apparently monomorphic. Here, then, as in certain sea-urchins, the female is apparently digametic, the male apparently homogametic. Further investigations must establish the fact whether the same reverse condition obtains with respect to an X-element in the case of the remaining four mammals of the first group.

Male and female sex (together with secondary sexual characters) appear to be hereditary in a manner similar to other organic characters, and may be regarded as a pair of "unit characters." Since the femaleness is associated with, or determined by, the presence of X-elements, the female (containing a pair of homologous X-elements) is presumably homozygous,[1] the male heterozygous for sex; or, in accordance with a more recent terminology, the female represents a duplex (but apparently recessive like a nulliplex) condition, the male a simplex (dominant) condition. Maleness is accordingly characterized by the absence, femaleness by the presence of a "determiner" (presumably an inhibitor), the X-element. The former is the negative or minus (lacks accessory, contributed to female at fertilization), the latter the positive or plus, condition. Sex inheritance conforms thus to the Mendelian scheme, in which maleness represents the DR and femaleness the RR condition; or, stated in terms of the presence (+) and absence (−) of an inhibiting accessory, or X-element, maleness is + −, and femaleness the ++ condition. Femaleness may therefore be plus or positive in an inhibiting sense; a germinal plus condition prevailing in the female, inhibiting the appearance of a positive somatic condition or male sex.

This modified Mendelian interpretation is suggested in part by an analogy with conditions in crosses between horned and hornless cattle, where the positive character (horns) is recessive to the negative or hornless character; meaning, according to the general interpretation, that hornlessness is determined by the presence of some determiner which prevents the development of horns. Similarly, femaleness may be due to the presence of the X-element, preventing the development of maleness.[1] It would be immaterial whether it were contributed by the male or by the female gamete. This idea is further suggested by, and seems in perfect accord with, the numerous observations indicating that femaleness is undeveloped maleness, *e. g.*, embryological facts; relatively female characteristics of male infants; the assumption of male secondary characters after spaying, or after the menopause; etc.

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[1] That is, in the group where the male germ-cells contain an X-element.

SUMMARY AND CONCLUSIONS.

Among the forms examined, including mongoose, cat, squirrel, pig, rabbit, white mouse, sheep, horse, mule, bull, and dog, heterochromosomes are apparently lacking in the male germ-cells of the first five, and present in the remainder. The available evidence favors more the interpretation in terms of a bipartite univalent or compound X-element than of an associated X and Y group (idiochromosomes).

In view of the fact that heterochromosomes have recently been reported in man (Guyer, double X-element; Gutherz, equal pair of idiochromosomes, or X- and Y-elements), rat (Guyer), armadillo (Newman and Patterson), guinea-pig (Stevens), and opossum and bat (Jordan), the evidence indicating similar elements in the above-enumerated group of six common mammals would seem to warrant the conclusion that sex-chromosomes are very generally present in mammals. Universality of presence seems vitiated for the present by the fact that in another group of five mammals, carefully studied, such elements seem apparently lacking. It might be assumed that such elements are actually present in the male germ-cells, but are so small or labile as to elude detection by ordinary methods, or do not present the usual morphology of heterochromosomes during the prophase stages. The unmistakable presence, however, of a "split-accessory" in the female germ-cells (primary oöcyte) of the cat, as recorded by Winiwarter and Sainmont, and the absence of any X-element in the male (confirmed by Gutherz, 1912), suggests very forcibly that sex-chromosomes are present in all mammals, generally in the male, exceptionally in the female. The same result would follow (that of numerical sex-equality) whether present in one or the other sex. If this hypothesis can be further sustained, it would seem cogently to reinforce the evidence for an essential sex-determining function of heterochromosomes. Interpreted in terms of Mendelian heredity-formulæ, in those mammals in which an X-element is present in the male, the female sex is homozygous, the male heterozygous. The facts would seem to fit the hypothesis that the accessory chromosome acts as a deterrent to the development of maleness; or more accurately, and in keeping with a quantitative interpretation of sex in a final analysis, the accessory with its egg-homologue (two X-elements) inhibits male-sex development, the single egg-homologue in males being insufficient to counteract the male tendency, thus giving origin to male individuals. In those instances where the female can be shown to be the heterozygote, one X-element, according to the hypothesis, may be assumed to be able to counteract the male tendency.

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[1] However, in the case of horn-heredity, the inhibitor, according to this explanation, is dominant; in sex-heredity, recessive. In cases where the female is the heterozygote, the condition might be parallel to that of horn-heredity. In these instances the female would be simplex, the male nulliplex. Recession of a duplex condition seems a contradiction in terms. The suggestion can have significance in only an approximate or general sense.

ADDENDUM.

The opportunity recently presented itself for the study of very favorable human material. I am indebted to Dr. H. T. Marshall, professor of pathology at the University of Virginia, for the specimen, a thin slice of testicle fixed in Zenker's fluid, and obtained at autopsy from a negro aged 38 years, who died as the result of drinking wood alcohol. The material is in excellent state of preservation. It was stained for study with iron-hematoxylin. Primary spermatocytes in early postsynaptic phases (text-figs. 1 to 9) are especially abundant and clear. Mitoses, however, both in spermatogonia and spermatocytes, are infrequent, and, though a sufficient number can be found at metaphase in the latter cells to furnish a fairly satisfactory opportunity for attempting chromosome counts, I can come to no definite conclusion concerning the actual number. My specimens of primary spermatocyte mitoses, however, compare very favorably with the illustrations given by Guyer and by Gutherz, every one of which I can practically duplicate.

An impartial judge of the figures must admit, I believe, that certainty is impossible at least within an error of two—and two makes all the difference between the presence of an accessory as urged by Guyer and denied by Gutherz. The inherent difficulty of an accurate count is indicated by the fact that the diploid chromosome number of the male cells has been given as 22 (Guyer), 24 (Flemming, Duesberg, Branca, Gutherz), 16 (Bardeleben), 18 (Wilcox), 33 or 34 (Wieman), and 47 (Winiwarter). It is significant, however, that Guyer, Gutherz, Duesberg, and Branca agree on 12 as the haploid (reduced) number; but Guyer, in contrast to the others, regards 2 of these as univalent accessories; also Guyer, Gutherz, and Winiwarter agree in claiming the presence of a heterochromosome; and Guyer and Winiwarter both report a dimorphism of spermatozoa, the former giving the number of chromosomes as 10 and 12, and the latter as 23 and 24.

It seems clear that a heterochromosome of some valency is present; it is equally clear that the true nature of this body can not yet be established on the basis of numerical relationships; the count is still uncertain as regards at least 2 chromosomes. Study of my material convinces me that the reduced number is not less than 12, but perhaps several more. As regards conclusions respecting the presence of an accessory chromosome drawn from a study of side views of metaphase spindles, it may be pointed out that Guyer's and Gutherz's figures are substantially alike; but Guyer interprets the appearance as demonstrating, Gutherz as contradicting, the presence of an accessory. My own figures occasionally show a double chromosome at one pole in advance of the main complex, and the evidence favors Guyer's interpretation rather than Gutherz's, but there is here nothing so striking as one sees in certain insects (*e. g.*, the phasmid, *Aplopus mayeri*) and the opossum. Strong evidence for the presence of a heterochromosome in man is given by Gutherz in his illustrations of early postsynaptic nuclei (text-figs. 2 to 6). But neither he nor Guyer seems to offer satisfactory evidence as to its composition from chromosome counts and behavior at metaphase. Both, moreover, regard it as a double structure. My own evidence confirms the findings of Guyer and Gutherz in so far as pertains to the actual presence and bipartite nature of a heterochromosome or X-element, and it emphasizes the additional points of similarity between this and undoubted sex-chromosomes, namely, frequent connection with the diplotene thread and close spatial relationship to nuclear wall (exception, text-fig. 3).

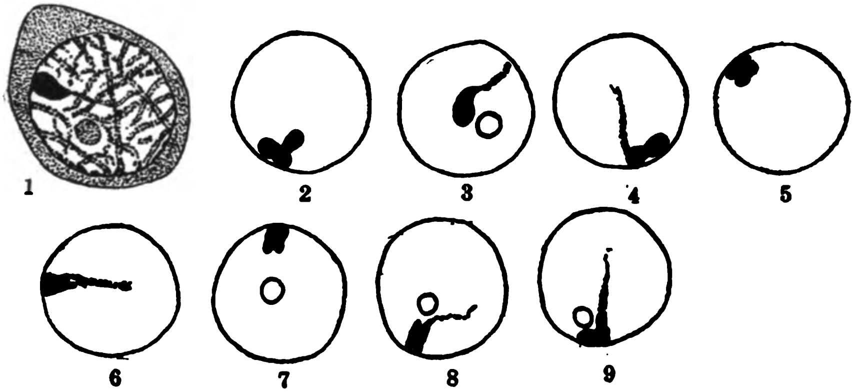

Fig. 1.—Human primary spermatocyte; nucleus in early diplotene post-synaptic phase, showing a deeply staining oval chromatin (chromosome) nucleolus on the nuclear wall and attached to one of the threads; the true nucleolus is a spherical, less deeply staining body, and only very rarely placed close to the nuclear wall. The cytoplasm is homogeneous, finely granular.

Figs. 2 to 9.—Outlines of nuclei at same stage to show the general form and location of the heterochromosome. It is almost invariably bipartite and attached to one of the threads, and on the nuclear membrane. The nucleolus when present is indicated in outline.

Judging from the illustrations, Winiwarter (1912) has had the advantage of possessing by far the best human material. His illustration (fig. 25) is practically a duplicate of my own of the postsynaptic diplotene (passing into the pachytene) phase. However, the later history of the accessory element as traced by Winiwarter is not at all stages perfectly clear. But figures 39 and 40 show pairs of sister spermatids, one pair with, the other without, a deep-staining nuclear body (accessory chromosome?). If this spermatid element actually represents, as seems very probable, the postsynaptic nuclear bipartite body, or heterochromosome, then cogent additional evidence here also accrues favoring the legitimate interpretation as a heterochromosome of the similar (structurally and tinctorially) peculiar postsynaptic nuclear element wherever found in mammals.

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DESCRIPTION OF PLATE.

Fixation: Flemming's strong solution. Stain: Heidenhain's iron hæmatoxylin.
Magnification: 1500 diameters.

Mongoose.

1. Spermatogonium; the nucleus contains three small plasmosomes.

2. Spermatogonium; the nucleus is in process of amitotic division.

3. Primary spermatocyte; the nucleus contains a centrally located plasmosome; the delicate chromatic reticulum consists of an apparently continuous single thread; leptotene-nucleus.

4. Bouquet stage; the slightly thickened spireme has aggregated in a close-meshed mass at the idiosome pole of the nucleus; the cytoplasm surrounding the idiosome contains pale mitochondria; synizesis, polarized amphitene.

5. Synapsis: the disentangling threads are pairing side by side; the nucleus is still approximately of the same size as in the resting spermatocyte, hence synizesis and synapsis signify activity (motion) on the part of the nuclear reticulum; synaptene-nucleus.

6. Postsynapsis; early prophase; diplotene-nucleus.

7 and 8. Later prophases; at no stage do heterochromosomes appear.

9 and 10. Side and polar views respectively of metaphase plates; no chromosome is conspicuous for unusual size or behavior; the number of chromosomes is approximately twenty-four, the haploid group.

11. Resting secondary spermatocyte.

12 and 13. Side and polar views respectively of metaphase plates; the number of chromosomes is approximately twelve, forming a hemioid group.

14. Spermatid; the cytoplasm has become filled with pale mitochondria.

15. Appearance of "sphere" and centrosome.

16 and 17. Appearance of axial filament and middle piece.

18 and 19. Formation of spiral filament by coalescence of mitochondria.

20 and 21. Face and profile views respectively of mature, living spermatozoa.

White Mouse.

22. Spermatogonium.

23. Primary spermatocyte, nucleus at leptotene phase; the bilobed nucleolus may be a pair of heterochromosomes.

24. Synapsis, polarized amphitene, bouquet or synaptene phase; the heterochromosome is apparently single.

25. Postsynapsis; early prophase; heterochrome (or accessory) conspicuous; pachytene-nucleus.

26 and 27*a*. Early and late prophase groups respectively, both including conspicuously the pair of heterochromosomes almost invariably situated on the nuclear wall and close to the idiosome; 26, diplotene-nucleus.

27*b*. Metaphase; one of the chromosomes has moved in advance of the others to one pole; this has a tetrad form and is still clearly bivalent; accordingly, it fails to divide in this mitosis and may represent the "accessory" of the growth stages.

Sheep.

28. Resting primary spermatocyte, showing the accessory at the idiosome pole; perhaps postsynaptic "confused stage" of Wilson.

29. Bouquet (synapsis) stage; the accessory chromosome is attached to a double thread, and invariably lies close to the nuclear wall.

30. Postsynapsis; early prophase, showing a bipartite accessory (or pair of heterochromosomes).

Horse.

31. Primary spermatocyte; the nucleus contains two irregular karyosomes and a bilobed chromatic nucleolus, probably a pair of heterochromosomes.

32. Synapsis stage, showing the heterochromosomes among the pairing threads close to the nuclear wall.

33. Postsynapsis, showing a tripartite X-element, the heterochromosome group; pachytene-nucleus.

Mule.

34. Resting primary spermatocyte, showing a divided, or paired, heterochromosome.

35. Synapsis stage, showing a heterochromosome.

36 and 37. Early and late prophase stages respectively, showing a pair of heterochromosomes.

Bull.

38. Postsynapsis, showing an accessory chromosome among the bivalent threads, close to wall and idiosome; diplotene-nucleus.

Dog.

39. Primary spermatocyte; presynaptic resting phase, showing plasmosome and bilobed monosome.

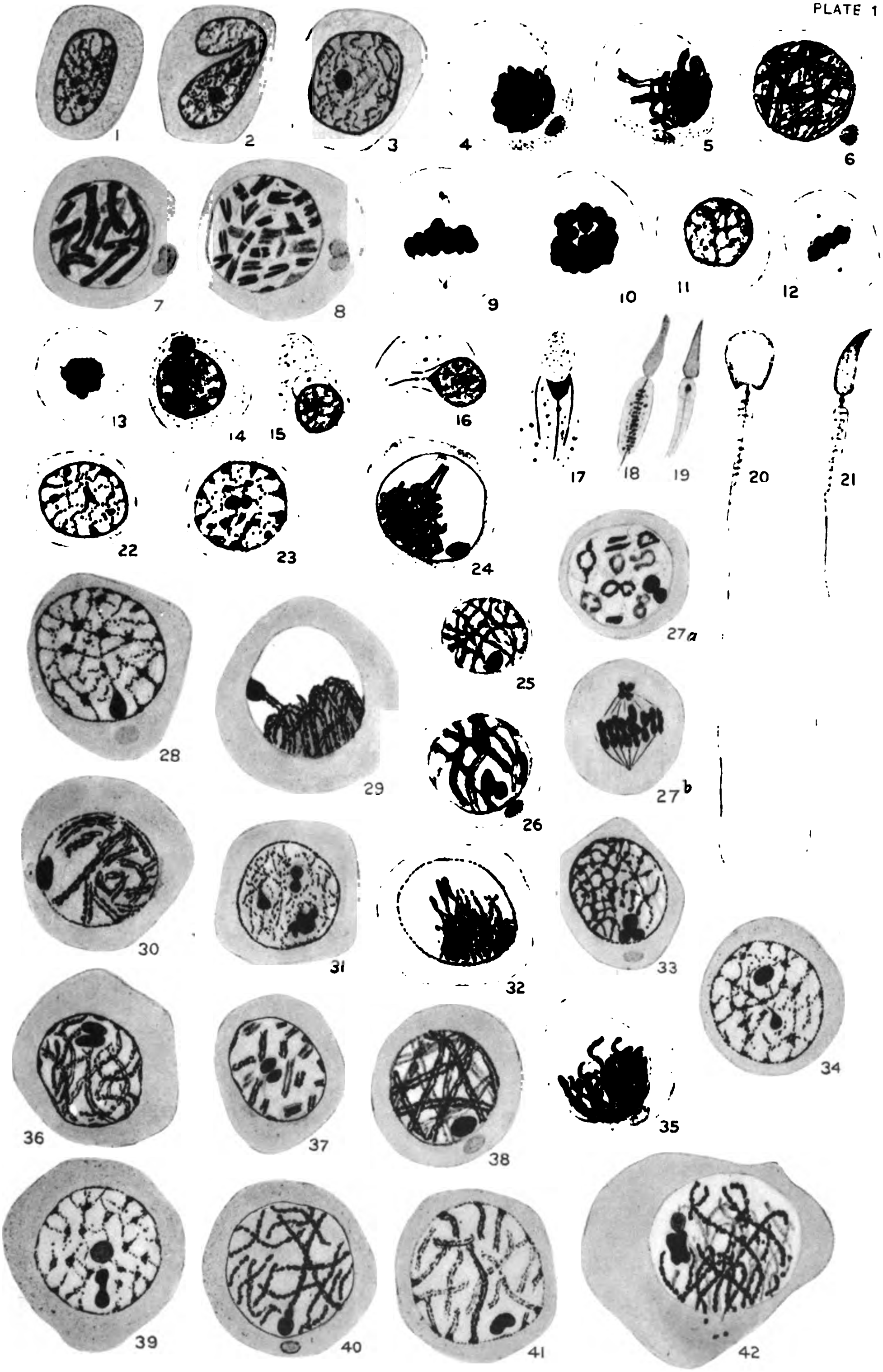
40. Postsynaptic phase, pachytene-nucleus; monosome attached to one of the threads.

41. Early prophase, diplotene-nucleus; chromosomes in form of long bivalent threads; monosome close to nuclear wall.

42. Oöcyte from ovary of 23-day cat. Synapsis. True nucleolus; monosome divided longitudinally (after H. von Winiwarter et G. Sainmont, fig. 43).

JORDAN

PLATE 1



XI.

THE BRYOZOA OF THE TORTUGAS ISLANDS, FLORIDA.

BY RAYMOND C. OSBURN.

Twenty-three text-figures.

THE BRYOZOA OF THE TORTUGAS ISLANDS, FLORIDA.

By Raymond C. Osburn.

In the summer of 1908 the writer had the privilege of spending the month of June at the Carnegie Institution Laboratory for Marine Biology, located on Loggerhead Key of the Tortugas Islands. Owing to the short time at my disposal the entire period was devoted to a close search for the bryozoa inhabiting the shallow waters about the reefs, on the piles of the old government dock on Garden Key, in the moat of old Fort Jefferson on the same key, and in dredging the shallow waters about the islands down to 22 fathoms. For much of the work a skiff or a small launch was used, and for the deeper dredging (10 to 22 fathoms) the schooner *Physalia* was employed. The Tortugas Islands are islets of a coral atoll and in the diversified bottom afforded by such a region a rather abundant bryozoa fauna was found.

Comparatively little work has been done on the bryozoa of the Florida and West Indian regions. Smitt's papers on the Florida bryozoa (1872–73) deal with 87 species collected by Count L. F. de Pourtales and afford the only extended record of the bryozoa of this region. Pourtales (1867), in a paper entitled "Distribution of the Fauna of the Gulf Stream at Great Depths," listed and described as new 7 species of bryozoa, 2 of which have been proved to be synonyms. Verrill, in his Bermuda papers, has listed 21 species from the Bermuda Islands. Levinsen, in his important work, "Morphological and Systematic Studies on the Cheilostomatous Bryozoa" (1909), records 6 species from the West Indian and Florida region, describing two of them as new. Otherwise this vast region remains untouched. As the collections of Pourtales were made in the deeper waters of the Florida region, it was presumed that careful collecting in the shallow waters would disclose the presence of a somewhat different fauna, a presumption well borne out by the results of my collecting. There is, indeed, a remarkable disparity between the list given by Smitt and that forming the basis of the present paper. Not only did 41 of the species described by Smitt fail to appear in the shallow waters, but 40 others, whose presence was not hitherto suspected in the Florida region, and many of them not even in America, were taken. This brings the list of the species at present known from Florida and West Indian waters up to 127.

By comparing with lists from other regions where the bryozoa have been carefully worked, it will be seen that the bryozoa fauna of the Tortugas and of the Florida-West Indian regions is fairly rich in species and fairly representative of tropical and semi-tropical regions. Careful collecting for a number of years in a limited region about Woods Hole, Massachusetts, and extending down to about 20 fathoms, yielded 83 species (see Osburn, 1912). Waters (1909–10) listed 73 species from the Red Sea. Norman (1909) records 139 species from Madeira and neighboring islands. This latter list is of special interest for comparison, since the Madeira bryozoa have been collected with some care for about 50 years and it may be supposed that it is fairly complete. In making comparison, however, it must be noted that the dredging about Madeira extended down to 200 to 300 fathoms, while those of the present list go only to 22 fathoms, so that to properly compare the Florida with the Madeira fauna the present list of shallow-water forms should be combined with that of Smitt.

The Tortugas bryozoan fauna (shallow water) presents a very different facies from that of Woods Hole at the same depth, since the two lists of 76 and 83 species, respectively, have only 13 species in common. The Woods Hole fauna is distinctly northern, while that of the Tortugas, at least for the shallow waters, is distinctly tropical. This is readily understood when we recall that the Tortugas are within 0.5 degree of the tropics, and that these islands receive, directly along their shores, the warm waters of the Gulf Stream which sweep up from the Caribbean Sea.

It is interesting to note in this connection that, of the 40 species here added to Smitt's list, 33 are limited entirely or chiefly to a tropical distribution.

The arrangement of the bryozoan species, genera, and families has undergone so much alteration in recent years, as a result of the studies of Norman, Waters, Calvet, and Levinsen, as to be wholly unintelligible to anyone except the systematist familiar with the changes of the last ten years. It is impossible that this should be otherwise, since the older classification was based almost entirely upon the structure of the skeleton of dead and dried material, without reference to the general morphology.

In the following list I have followed as far as possible the classification adopted by Levinsen (1909). In the cases of a few imperfectly understood species the older genera have been retained as "catch-alls." Thus the genera (?) *Lepralia* and *Phylactella* have been used in the old Hincksian sense for certain species which, with more complete knowledge, must undoubtedly go elsewhere.

The figures were drawn by Mr. S. Shimitori, except figures 7, 11, 22, and 23, which are the work of Mr. H. Murayama.

ENTOPROCTA.

GENUS PEDICELLINA Sars, 1835.

Pedicellina cernua (Pallas).

> OSBURN, 1912, p. 213, Synonymy and previous records of the occurrence of this species on the American coast.

Apparently not common, but several small colonies were taken on the piles of docks and dredged at 10 fathoms.

GENUS BARENTSIA Hincks, 1880.

Barentsia discreta (Busk).

BUSK, 1886, p. 44 (*Ascopodaria discreta*).—JULLIEN, 1888, p. 13 (*Pedecellina australis*).—(?) VERRILL, 1900, p. 594 (*Barentsia timida* sp. nov.).—WATERS, 1904, p. 99.—OSBURN, 1912, p. 214.

This species, first described from Tristan da Cunha in the *Challenger* reports, is now known to be widely distributed, as follows: Tristan da Cunha, 100 to 150 fathoms (Busk); China Sea, 27 fathoms (Kirkpatrick); Cape Horn, 26 fathoms (Jullien); Ile Londonderry, Magellanes, Chile (Waters); Woods Hole, Massachusetts (Osburn); Beaufort, North Carolina (Osburn); Tortugas, Florida (Osburn).

If Verrill's *Barentsia timida* from the Bermudas is the same species, as I strongly suspect, this will add another locality. Verrill states in regard to his *B. timida* that it is closely allied to *B. discreta*, but that the latter has a shorter and more annulated basal cylinder and also several annulations of the stem below the base of the cup. In my experience, the annulations of the stem are of little importance, if any, as there is much individual variation which may represent only differences in contraction at this point.

The individuals are small and the species may readily escape observation. It appears only once in my collection made at the Tortugas, being dredged in 18 fathoms directly north of the island on a bottom of coral mud; several fragments of colonies attached to shells.

CYCLOSTOMATA.

GENUS CRISIA (PART) Lamouroux, 1816.

? Crisia denticulata (Lamarck).

LAMARCK, 1816, p. 137 (*Cellaria denticulata*).—STIMPSON, 1853, p. 18.—SMITT, 1872, p. 4 (*Crisia eburnea*).—JELLY, 1889, p. 73.—HARMER, 1891, p. 136.—VERRILL, 1879, p. 28; 1900, p. 592.—WHITEAVES, 1901, p. 110.—OSBURN, 1912, p. 216.

Numerous small colonies were taken at from 10 to 15 fathoms in various places about the island, attached usually to sponges and shells. Smitt records the species as *C. eburnea* from 7 to 60 fathoms. Stimpson (1853), Verrill (1879), and Whiteaves (1901) have recorded it from New England to Canada, though these records are questionable on account of failure to consider the oœcia, and the writer has recorded it questionably from Woods Hole, Massachusetts (oœcia wanting). Verrill has reported it as common at Bermuda.

Harmer (*l. c.*), referring to Smitt's Florida record, states: "I do not feel certain that the form described is really identical with *C. ramosa*, although it can hardly be regarded as *C. denticulata*," and suggests that it may be Stimpson's *C. cribraria*. It does not seem to agree with the latter species, however, in any essential point (see Osburn, 1912, p. 215, for redescription of *C. cribraria*), nor does it agree with *C. ramosa*, in which the radical fibers have long internodes with yellow or colorless joints. It does agree with *C. denticulata* in having short internodes in the radical fibers and the joints of these fibers, as well as those of the zoarium, conspicuously jet-black. As the form of the colony and of the zoœcia agree fairly with *denticulata*, I retain that name for the species. It is unfortunate that ovicells are wanting, so that it is impossible to make a positive determination, for, in spite of the number of times the species has been recorded from North America, there seems to be not one of these records based on an unquestioned identification.

GENUS LICHENOPORA Defrance, 1823.

Lichenopora hispida (Fleming).

FLEMING, 1829, p. 530 (*Discopora hispida*).—JELLY, 1889, p. 134, synonymy.

A very minute specimen was taken on an alga at 2 fathoms and placed with some question in this species. A well-developed colony taken by Dr. Paul Bartsch at Biscayne Key, Florida, has recently been examined, and after comparison there seems to be no doubt as to the identity of the Tortugas specimen. The species hitherto has not been noted in the Florida or West Indian regions.

CHEILOSTOMATA.

GENUS AETEA Lamouroux, 1812.

Aetea truncata (Landsborough).

LANDSBOROUGH, 1852, p. 288 (*Anguinaria truncata*).—JELLY, 1889, p. 5, synonymy and references.—CORNISH, 1907, p. 75.—NORMAN, 1909, p. 283.

Common in shallow water and down to 5 fathoms, creeping over shells and seaweed. It has been recorded at Canso, Nova Scotia (Cornish, *l. c.*).

Aetea sica (Couch).

COUCH, 1844, p. 102 (*Hippothoa sica*).—JELLY, 1889, p. 5 (*Ætea recta*) synonymy and references.—WATERS, 1909, p. 129 (*Ætea recta*).—NORMAN, 1909, p. 283.

Tortugas at 10 fathoms on shells. Not previously noted in American waters.

Some authors prefer to consider this only a variety of *Ætea anguina*. The latter has not been taken in the Florida region, though it is common northward from Beaufort, North Carolina.

GENUS BUGULA Oken, 1815.

Bugula neritina (Linné).

LINNÉ, 1758, p. 38 (*Sertularia neritina*).—VERRILL, 1878, p. 304 (*Acamarchis neritina*); 1900, p. 588.—ROBERTSON, 1905, p. 266.—WATERS, 1909, p. 135.

This widely distributed warm-water species is abundant in the shallow water, growing on piles, shells, seaweed, etc. Colonies 1.5 inches in height with oœcia abundantly developed were taken on the bottom of a skiff which had been in the water only from May 1 to June 23.

Verrill has recorded the species for the Bermuda Islands and from Fort Macon, near Beaufort, North Carolina. Otherwise it has not been noticed on the American side of the Atlantic. Robertson found it common on the Pacific coast as far north as Monterey Bay, California.

Bugula neritina var. minima Waters.

WATERS, 1909, p. 136.

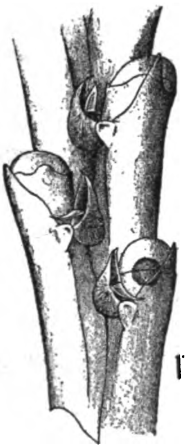
One small colony with ovicells abundantly developed and with numerous avicularia (as described by Waters in Red Sea specimens) was taken at a depth of 8 fathoms. Previously known only from the type locality. This variety is distinguished from the typical *neritina* by the smaller size of its zoœcia and by the presence of avicularia situated near the lower end of the zoœcia.

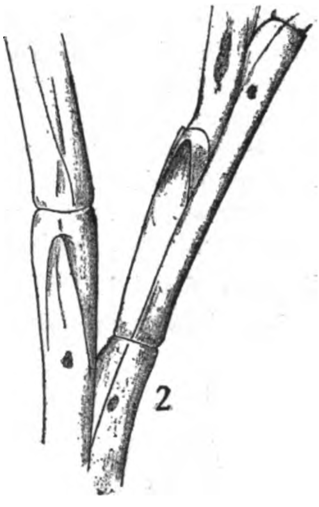
Bugula flabellata (Gray).

GRAY, 1847, p. 106 (*Avicularia flabellata*).—SMITT, 1872, p. 18.—VERRILL AND SMITT, 1873, p. 711.—VERRILL, 1879*b*, p. 189; 1880, p. 29 (*Bugula flustroides*).—OSBURN, 1912, p. 225.

A single small colony of this species, attached to *Retepora atlantica*, was taken at a depth of 12 fathoms. The ovicell is more widely opened than in Woods Hole specimens. A very widely distributed species, occurring northward on our coast to Maine. Miss Robertson found it at San Diego, California.

Bugula microœcia n. sp. (Figs. 1 to 3.)





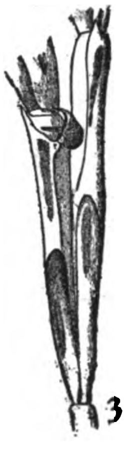


FIG. 1.—*Bugula microœcia* n. sp. Front view of portion of branch, showing details. Upper two oœcia not quite complete. Lower one complete with embryo.
FIG. 2.—The same. Portion of main branch showing joints. Degenerate polypides are present.
FIG. 3.—The same. Portion of stem higher up, showing less-modified zoœcia. Remains of degenerate polypides within.

Zoarium delicate, composed of a central stalk with long, narrowly flabellate branches arising in an irregular dichotomous fashion from the main stem. The stalk, as well as its branches, consists of very much elongated and modified zoœcia arranged biserially. That these are really zoœcial in character is shown by the fact that they contain polypides and occasionally one of them even bears an avicularium. Notwithstanding this, definite joints appear at the bifurcations of the main and all the accessory branches. These conditions show an interesting connecting link between the *Bugula* and *Stirparia* types of stems. From the lower stem zoœcia arise numerous, strong, radical fibers.

The ordinary zoœcia, which are arranged biserially, are long and slender and expand but little toward the tip; the frontal aperture extends almost to the base and faces somewhat toward the axis of the branch; a strong spine is situated at the distal outer edge; near the distal end, at the point where the outer wall curves farthest over the frontal area, is situated a short, stout avicularium with a strongly decurved beak; the avicularium is set upon a stalk so short as to be almost wanting.

The oœcium is hemispherical, very small, and set very low down, partially hidden by the spine, so as to be very inconspicuous; it is thin and transparent, with a slightly arched aperture. Many of these contain embryos in various stages of development.

Taken at 18 fathoms on a bottom of coral mud, several colonies attached to shell fragments; the largest 2.5 inches in height. Small fragments were also found decorating the legs of crabs (*Hyas* sp.?) at the same depth.

Bugula caraibica Levinsen.

LEVINSEN, 1909, p. 104, pl. III, figs. 2A–2N.

This species, recently described from St. Croix, Danish West Indies, is common at the Tortugas on the piles of the government docks, and colonies 1.5 inches high were taken on the bottom of a skiff which had been in the water only from May 1 to June 23, 1908.

It is one of the most conspicuous species of the region, growing in loose tufts of a fine purple color to the height of 4 to 6 inches. Levinsen does not recognize *Stirparia* as a separate genus, but if the species with a jointed stalk are to be separated, *caraibica* will fall in that genus.

Bugula armata Verrill. (Figs. 4 and 5.)

VERRILL, 1900, p. 588 (*Bugula* [*Caulibugula*] *armata* n. sp.).

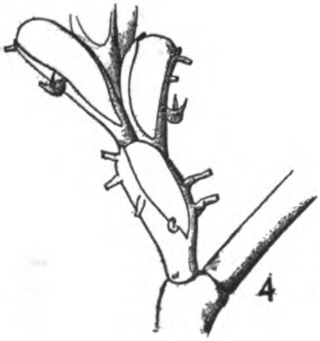


FIG. 4.—*Bugula armata* Verrill. Modified zoœcia at base of branch, showing arrangement of spines and position of avicularia.

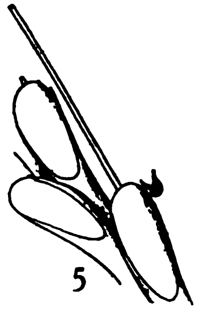


FIG. 5.—The same. At tip of same branch, showing character of spines and position of avicularium.

Verrill has described from Bermuda a species which I take to be the same as one occurring at Tortugas. His description is as follows:

> A much more delicate, white *Bugula*, consisting of diverging, fan-like branches attached to the alternate sides and to the tip of slender jointed stems, sometimes having alternately a long joint and a very short joint, but more frequently the short joint is lacking and the end of the joint is swollen, as in *Stirparia*. There are usually 2 or 3 annulations at the base of each main branch and these arise just below the internodes. Many of the cells have a slender, distal vibraculum or sometimes two. It should doubtless form the type of a new genus or subgenus (*Cauli-*

***bugula*) intermediate between *Bugula* and *Bicellaria* on account of its articulated spines or vibraculum, and related to *Stirparia* by its jointed stem. It may be named *Bugula* (*Caulibugula*) *armata*. Its zoœcia are oblong and biserial, alternate. The pedicellariæ are on short pedicels, large, lateral not numerous.**

Verrill in the above description overlooked some interesting and important points. He makes no mention of radical fibers which originate at the lower ends of the internodes of the stalks. He does not mention the oœcium, which is subglobose with a rather wide opening directed toward the zoœcial axis. The oœcium is attached by a narrow stalk to the outer edge of the zoœcial aperture near the distal extremity and is turned sidewise at a right angle to the zoœcial axis.

The avicularia ("pedicellariæ" of Verrill) show a peculiar distribution. The basal zoœcium bears no avicularium, but the zoœcia immediately above have the avicularia situated on the side about half-way along the aperture. The next few cells in order have the avicularium farther and farther removed toward the distal end, and in all zoœcia farther out on the branches the avicularium is on the distal outer edge of the aperture. This is a feature which I have never seen exhibited by any bryozoa. There are one or two ong spines, jointed at the base, on the distal end of the zoœcium (sometimes wanting). Verrill errs in calling these "vibracula," a term which should be applied only to modified avicularia with elongate mandibles. The basal zoœcium of the main branches arising from the stem differs from the others in having usually 6 spines surrounding the aperture, 4 on the outer, 2 on the inner edge, a feature exhibited by *Stirparia occidentalis* (Robertson, 1905, pl. XIII, fig. 73).

I can see no reason for erecting a new genus for this species. The jointed stalks, swollen at the nodes, seem sufficient to place it in *Stirparia* if that name were to be maintained for the Bugulas with jointed stalk. The jointed spines do not necessarily relate the species to *Bicellaria*, as this feature is present in some species of *Bugula* and *Stirparia*.

Taken on a number of occasions at 8 to 10 fathoms, growing in a sprawling fashion over sponges. Found also on the legs of spider crabs at 10 to 18 fathoms, and dredged on a bottom of coral mud at a depth of 18 fathoms.

GENUS BEANIA Johnston, 1838.

Beania mirabilis Johnston.

JOHNSTON, 1847, p. 372.—JELLY, 1889, p. 17, for further reference.—ROBERTSON, 1905, p. 276.

Several fragments of colonies on the legs of a crab (*Hyas* sp.) taken at 18 fathoms. This striking and well-known species has not before been noticed on the American side of the Atlantic.

Beania intermedia Hincks.

HINCKS, 1881*a*, p. 133 (*Diachoris intermedia*).—WATERS, 1906, p. 15; 1909, p. 137.

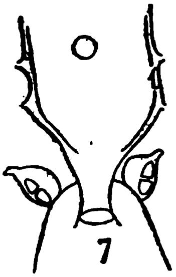
At 5, 10, and 15 fathoms, sprawling over hydroids and other bryozoa, and on shells. Very small colonies consisting of only a few cells. The simple, spineless character of the zoœcium easily serves to distinguish it from others of the genus.

The species is entirely southern in its distribution, being recorded from Tasmania (type locality), Australia, Chatham Island, Ganjam coast, and the Red Sea. The present record therefore adds the species to the Atlantic fauna and greatly increases the known range.

Hincks described the species as having spines on each side. Waters (1909) remarks that they are absent in Red Sea specimens, but sometimes present in those from Chatham Island. There is no indication of them in my material.

Beania cupulariensis n. sp. (Figs. 6 and 7.)

Small, straggling colonies growing attached to the under side of *Cupularia guiniensis*. The individual cells are fairly large (about as in *B. mirabilis*). On each side of the flattened frontal area there are about 6 (5 to 7) slender marginal spines which curve somewhat over the area. At the distal end 2 smaller spines project forward. At each side of the aperture is placed an avicularium situated on a short stalk on margin of cell; as far as observed, these are always paired. The form is short, the beak strongly decurved. The zoœcium arises from the preceding one of the series, near the distal part of the dorsal surface, by a tubular stalk so short that the top of one zoœcium projects over the base of the next in series. A branch arises about midway of the zoœcium near the lateral margin. Here again the tubular portion is very short and, as far as observed, only one branch arises from a zoœcium—never in pairs. There seem to be no other processes arising from the zoœcium and many of the cells have no tubes, branches, or other processes arising from them, except that of the zoœcium next in the series and a dorsal process for attachment.



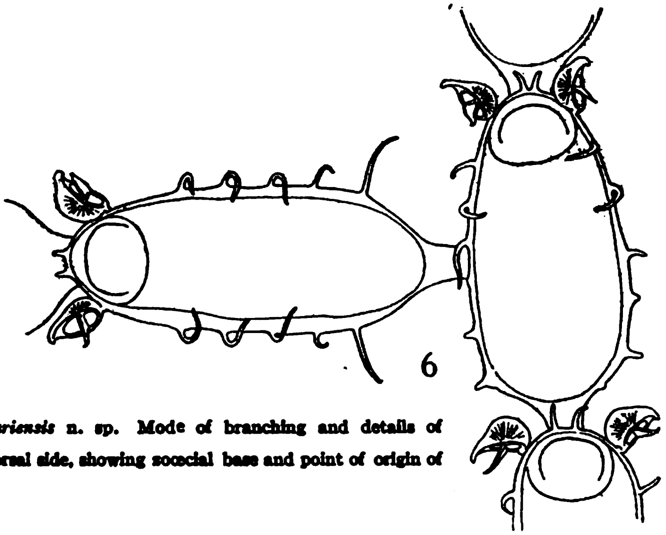


FIG. 6.—*Beania cupulariensis* n. sp. Mode of branching and details of zoœcium.
FIG. 7.—The same. Dorsal side, showing zoœcial base and point of origin of dorsal fiber.

The colony appears to be very loosely attached to the *Cupularia* by processes from the dorsal sides of the cells. The species occurred at various depths—in fact, wherever this species of *Cupularia* was found, at from 10 to 22 fathoms, but was found nowhere else. Usually only a single colony was found on one *Cupularia*, but occasionally on a large specimen two or three colonies would occur.

GENUS SYNNOTUM Hincks, 1886.

Synnotum aviculare (Pieper).

PIEPER, 1881, p. 43 (*Gemmellaria avicularis*).—HINCKS, 1886, p. 257.—ROBERTSON, 1905, p. 286.

This well-known species is abundant about the islands, growing especially on shells and sponges at a depth of 8 to 10 fathoms. It has not before been recorded from North America, except on the Pacific coast, where Miss Robertson found it at San Pedro and San Diego, Southern California. Hincks (1886) described the sessile avicularia as alternating on Adriatic specimens. Robertson states that they appear on every pair in specimens from California. In the Florida material in my collection the alternate condition is the rule, but occasionally sessile avicularia appear on successive pairs of zooecia. Besides the above localities the species is now known from the Mediterranean and Red Seas, South Africa, Australia, and the Andaman Islands.

GENUS NELLIA Busk, 1852.

Nellia oculata Busk.

BUSK, 1852, p. 18.—LAMARCK, 1816, p. 135 (*Cellaria tenella*).—SMITT, 1873, p. 3.—JELLY, 1889, p. 94 (*Farcimia tenella*, references and synonymy).—WATERS, 1909, p. 167 (*Farcimia oculata*).—LEVINSEN, 1909, p. 120 (*Nellia tenella*).

Abundant at 10 to 18 fathoms, frequently attached to sponges or occasionally to shells. The usual height of the colonies is 0.5 to 1 inch. One large, branched colony was taken, measuring 4 inches across and of equal height. Smitt recorded the species from 13 to 138 fathoms. It has also been recorded from Texas and St. Thomas, West Indies (Levinsen), and is widely distributed in the Atlantic, Pacific, and Indian Oceans down to a depth of 550 fathoms.

Waters (1909, p. 167) objects to the use of Lamarck's name *tenella* on the ground that the short description given by Lamarck is not diagnostic and "might be applied to several species or even genera."

GENUS SCRUPOCELLARIA van Beneden, 1844.

Scrupocellaria cornigera (Pourtales).

POURTALES, 1867, p. 111 (*Canda cornigera*).—SMITT, 1872, p. 14 (*Cellularia cornigera*).—JELLY, 1889, p. 61 (*Cellularia cornigera*).

One small specimen of this species was taken at 10 fathoms and several others at 15 fathoms. The radical fibers are characteristically armed with stout, retrorse spines, as figured by Smitt. This alone is not sufficient for determining the species, as Busk has noticed serrate radicals in *S. ferox*

Busk and *S. macandrei* Busk, and Waters has named a species from the Red Sea *S. serrata* on account of this character. Florida specimens of *S. cervicornis* Busk show similar spines occasionally on the basal radicals. *S. cornigera* is a very delicate species and the radical fibers are transparent and thread-like, and this fact alone will serve to distinguish it from *S. cervicornis*.

Pourtales took the species at 270 fathoms, so it has a rather wide range in depth and temperature.

Scrupocellaria cervicornis Busk.

BUSK, 1852, p. 24.—SMITT, 1872, p. 14 (*Cellularia cervicornis*).—VERRILL, 1900, p. 594.

Occurs commonly about the islands, from low water to 18 fathoms, growing on piles of docks, attached to stems of gorgonias, etc. Colonies about 0.5 inch in height, with oœcia containing eggs, were taken from the bottom of a skiff on June 23, which had been in the water only since May 1. Recorded by Smitt from 7 to 17 fathoms.

The radical fibers, which are abundant at the base of the colony, are usually smooth, but in a few cases they bear retrorse spines like those of *S. cornigera*. In such cases the species are easily distinguished by the fibers alone, as they are much coarser in *cervicornis*. The cervicorn spines on the outer distal extremity of the zoœcia are well marked.

GENUS CANDA Lamouroux, 1816.

Canda caraibica Levinsen.

LEVINSEN, 1909, p. 142.

This species has been recently described by Levinsen, who gives no further indication of its distribution than is embodied in his remark "plentiful material of a West Indian species." Levinsen suggests the probability that this species is identical with *C. simplex* Busk, but being unable to determine this point he gives a new name to the West Indian material.

Several colonies were taken by the writer at the Tortugas at a depth of 15 fathoms, attached to shells. The largest of these was not much over 0.25 inch in height and no ovicells nor avicularia were present. The absence of an opercular spine and the length of the membranous frontal area (two-thirds of the whole zoœcium) show this form to be that described by Levinsen. The vibraculum in some cases is longer than the width of the branch.

The other Florida species of this genus, *C. retiformis* Pourtales (1867, p. 110), which Levinsen wrongly attributes to Smitt, is quite distinct from *C. caraibica*, since it possesses a broad opercular spine, the membranous area is but little more than half as long as the zoœcium, and there are differences in the vibracula. *C. retiformis* was not found at the Tortugas.

GENUS MEMBRANIPORA Blainville, 1834.

Membranipora membranacea (Linné).

LINNÉ, 1766–68, p. 1301 (*Flustra membranacea*).—PACKARD, 1867, p. 274.—ROBERTSON, 1908, p. 267.—NORMAN, 1909, p. 286.

One colony on the bottom of a skiff. In every respect this colony agrees with European specimens in my collection, except that there is a very slight extension of the calcification over the area at the base of some of the zoœcia. This is so slight, however, that I can not consider it of specific value. The species was not listed for Floridian waters by Smitt, nor was it taken by the writer at Woods Hole, Massachusetts, in several years collecting.

Packard recorded it from the Labrador coast. It is a common European species, occurring as far south as Madeira (Norman). It is also found in Australia and New Zealand, and Miss Robertson (1908) states that on the Pacific coast of North America it occurs from California to Alaska.

? Membranipora lacroixii (Audouin).

AUDOUIN, 1826, p. 240 (*Flustra lacroixii*).—BUSK, 1852–4, vol. 2, pl. 1.—DAWSON, 1859, p. 256.—PACKARD, 1867, p. 8.—SMITT, 1873, p. 18.—JELLY, 1889, p. 162 (*M. reticulum*).—WATERS, 1898, p. 697.—WHITEAVES, 1901, p. 97.—ROBERTSON, 1908, p. 261.—OSBURN, 1912, p. 227.

Taken in drift incrusting the carapace and legs of a large crab, and at 8 fathoms on shell. The specimens taken at Tortugas are much more heavily calcified than those from Massachusetts and between the zoœcia are numerous, roughly triangular prominences with triangular, membranous areas, as figured by Busk (l. c., pl. 1), while these are almost wanting in the Massachusetts specimens. Examination of the reverse side of the zoœcium, however, reveals the transparent circles and the irregular projections described by Waters (l. c.).

There has been much difference of opinion concerning this species and there can be but little certainty in regard to many earlier records, such, for example, as those of Dawson and Packard. Smitt's figures of Florida specimens seem, without question, to represent the same species as my own, though the dorsal surface is not shown. The range on the eastern coast of North America is from Florida to the Gulf of St. Lawrence, or to Labrador, if Packard's record is valid. Elsewhere it is known from Europe, New Zealand, and from California northward to Alaska.

Membranipora tehuelcha (d'Orbigny).

D'ORBIGNY, 1839, pl. 17 (*Flustra tehuelcha*).—BOSC, 1802, p. 118 (*Flustra tuberculata*).—JELLY, 1889, p. 167 (*M. tehuelcha*) and p. 168 (*M. tuberculata*).—WATERS, 1898, p. 674.—VERRILL, 1900, p. 594 (*Biflustra dentata*).—NORMAN, 1909, p. 286 (*Membranipora tuberculata*).—OSBURN, 1912, p. 231.

This species is found on *Sargassum bacciferum* (gulfweed) wherever it floats. It is abundant at the Tortugas. Verrill notes its occurrence at Bermuda, under the name of *Biflustra dentata*, as "common on Sargassum found upon the beaches." Smitt did not record it for the Florida region.

Norman prefers to use Bosc's older name for this species on the ground that, while his description means nothing, Bosc stated that it occurs "en immense quantité sur les fucus nageans dans l'Atlantique." As I am not aware that, under the rules of nomenclature, the habitat alone is considered a sufficient diagnosis, I prefer to follow Waters in using d'Orbigny's name.

Membranipora irregularis d'Orbigny.

D'ORBIGNY, 1839, p. 17, pl. VIII, figs. 5 and 6.—SMITT, 1873, p. 8, pl. II, fig. 63.—JELLY, 1889, p. 151, for synonymy.—WATERS, 1904, p. 31.

Incrusting shells at 8, 15, 18, and 22 fathoms. Smitt's figure is very satisfactory and there can be no doubt that the present species is the one which he figured as taken at 60 fathoms. The granulation of the border varies with the amount of calcification. Oœcia are present in some numbers. In younger stages these are quite prominent, but with later calcification they become included in the general crust.

Membranipora savartii (Audouin).

AUDOUIN, 1826, p. 240 (*Flustra savartii*).—SMITT, 1873, p. 20 (*Biflustra savartii*).—WATERS, 1909, p. 137.

One small, branching specimen 0.5 inch in height was collected at a depth of 10 fathoms. The broad, internal, basal denticle figured by Waters for Red Sea specimens is the best means of determining the species. Recorded by Smitt, 29 fathoms.

GENUS CUPULARIA Lamouroux, 1821.

Cupularia guiniensis Busk.

BUSK, 1854, p. 98.—BUSK, 1859, p. 87 (*C. canariensis*).—SMITT, 1873, p. 10 (*Membranipora canariensis*).—NORMAN, 1909, p. 289, synonymy.

Abundant at 10 fathoms, especially on soft bottom with algæ. The umbrella-shaped disks range in size from 0.125 to 0.75 inch in diameter. The color of living colonies is horn brown, due mainly to the chitinous bristles which form the mandibles of the avicularia. When touched the bristles stand erect for some time.

Recorded by Smitt from Pourtales's collections from 10 to 44 fathoms. Smitt listed this species under *Membranipora* because of the character of the zoœcium, holding the zoarial characters as of no importance. Just why he should have retained the related *C. lowei* and *C. doma* in *Cupularia* is not certain, but probably on account of the greater amount of calcification of the front wall.

The under surface of the colony is usually clear and free of any attached animals or plants, except that in numerous cases there occurs, closely attached, a new species of *Beania*, which I have named *B. cupulariensis*.

Cupularia lowei Busk.

BUSK, 1854, p. 99.—SMITT, 1873, p. 14 (*C. umbellata* Manzoni).—VERRILL, 1878, p. 305 (*C. umbellata*).—NORMAN, 1909, p. 290.

Only two specimens, both dead, were taken, one at 12 and the other at 22 fathoms, in company with *C. guiniensis*. Each was about 0.25 inch in diameter.

Recorded by Smitt for 29 fathoms and also in 7 fathoms off the mouth of the Cape Fear River. Smitt regarded *C. johnsoni* Busk (syn. *C. doma* d'Orbigny) as a highly calcified form of *C. lowei* (*umbellata*), but later investigations do not support this view.

Verrill (l. c.) recorded the species with doubt from one worn specimen taken at Fort Macon, near Beaufort, North Carolina. As the writer has taken specimens at the same locality, Verrill's record may be considered good.

GENUS CRIBRILINA Gray, 1848.

Cribrilina floridana (Smitt).

SMITT, 1873, p. 23 (*Cribrilina figularis* and local variety *floridana*).—JELLY, 1879, p. 66 (calls attention to fact that *C. figularis* of Smitt is not Johnston's species, and lists *C. floridana* as a distinct species).

A number of small colonies of this species taken at 5 to 15 fathoms on shells. Smitt recorded it from 29 to 42 fathoms. It is not known to occur outside of the Florida region.

No ooecia nor avicularia are developed on my specimens. The posterior margin of the orifice is straight, and the orifice is completely filled by the semicircular operculum, which is well chitinized and of a brownish color. There are two short, stout oral spines and in young zooecia there is frequently a third median spine.

There is a possibility that this species may belong to the genus *Puellina* of Jullien as amended by Levinsen (1909, p. 159), but the immature colonies in my collection seem rather to belong to *Cribrilina* in the strict sense.

GENUS ARACHNOPUSIA Jullien, 1888.

Arachnopusia monoceros (Busk).

BUSK, 1854, p. 72 (*Lepralia monoceros*).—JELLY, 1889, p. 67, synonymy.—LEVINSEN, 1909, p. 160 (discusses relationship and adopts Jullien's genus *Arachnopusia* with emendations).

A single specimen of this widely distributed species was taken at 5 fathoms, incrusting a shell. The colony was small, measuring only 0.125 inch across, but the zooecia ranged in development all the way from the ancestrula to adults with ovicells and from these to marginal individuals in various conditions of calcification. The species has not before been taken in North American waters.

GENUS SMITTIPORA Jullien, 1881.

Smittipora abyssicola (Smitt).

SMITT, 1873, p. 6 (*Vincularia abyssicola*).—JELLY, 1889, p. 253, synonymy and references.—HINCKS, 1881, p. 155; 1887, pp. 161 and 164.

Found at low water and down to 15 fathoms on shells, bases of gorgonias, etc. Smitt recorded two specimens taken by Pourtales, one at 68 fathoms, the other at 450 fathoms. The bathymetrical range of the species, from low water down to nearly 0.5 mile, is worthy of note.

Only the incrusting phase was seen, with no evidence of the erect stems described and figured by Smitt in one colony. The family relations of this genus are very uncertain. Hincks (1881, p. 155) placed it in the Microporidæ, but later (1887, p. 154) he removed it from this family on account of "the double layer of the ectocyst," and placed it in the Steganoporellidæ. As we understand the latter family at present, this genus can not be included in it. It will require a careful study of sectioned material to determine the real position of the genus.

GENUS STEGANOPORELLA Smitt, 1873.

Steganoporella magnilabris (Busk).

BUSK, 1854, p. 62 (*Membranipora magnilabris*).—SMITT, 1873, p. 15 (*Steginoporella elegans*).—VERRILL, 1900, p. 594 (*Steginoporella elegans*).—HARMER, 1900, pp. 279–286 (revision, synonymy, and detailed description).—LEVINSEN, 1909, pp. 167–168 (discussion of family and genus).

Common in low water to 15 fathoms. Beautifully convoluted, frill-like colonies were taken on piles and on shells, sponges, coral, etc., at 12 to 15 fathoms. The erect frill-like extensions are sometimes 2 inches high and 3 inches broad. These are bilaminate and are sometimes tubular in form. The color in life varies from pink to reddish brown.

Smitt recorded the species in Pourtales's collections from 15 to 37 fathoms; Bermuda (Verrill); other distribution given by Harmer (l. c., p. 280).

Steganoporella connexa Harmer.

HARMER, 1900, p. 254.

Dead colonies of what appears to be this species were taken incrusting shells at a depth of 12 fathoms. The opercula are all lost from the colony, so it is impossible to make use of this important structure for identification. The character of the calcification, with the junction of the median process with the sides of the zoœcium to form a pair of opesiulæ or foramina, is similar to that described and figured by Harmer. Since this connection of the median process with the zoœcial walls is known only in *S. connexa*, I refer my specimens to that species. The other points, as far as I can make them out on the dead specimens, agree with *connexa*, except perhaps in the matter of size, since the zoœcia in my specimens are smaller than described by Harmer. There is much variation in the matter of size, however, a condition also stated by Harmer to occur in *connexa*. Only one form of zoœcium can be distinguished.

The species is known from a single locality, "John Adams Bank," the location of which is somewhat in doubt, but Harmer seems to feel satisfied that it is south of Abrolhos Island off the coast of Brazil.

GENUS THALAMOPORELLA Hincks, 1887.

Thalamoporella rozierii (Audouin).

AUDOUIN, 1826, p. 239 (*Flustra rozierii*).—(?) SMITT, 1873, p. 16 (*Steginoporella rozierii*).—LEVINSEN, 1909, p. 181 (discusses at length, with five varieties).

Found on shell in drift and at 10 fathoms on sponges. Only small, unilaminar colonies without oœcia were taken. Smitt found only a single, small specimen in Pourtales's collections. His description of this specimen is brief and his figure (pl. 4, fig. 102) does not show the avicularium nor the spicules, so I include it in the above synonymy with some doubt. I have received from Professor A. E. Verrill a fine, erect, convoluted, bilaminate colony taken on the west coast of Florida. Levinsen (l. c., p. 183) records the variety *labiata* from Jamaica and my Florida specimens agree fairly closely with this variety.

Thalamoporella granulata Levinsen.

Levinsen, 1909, p. 188.

In the drift were found several dead specimens incrusting shells and calcareous algæ. The sinuated character of the orifice with the strong lateral denticles; the nearly equal opesiulæ reaching the marginal wall and with granulated edges; the dorsal opesiular outgrowths; the granulated character of the zoœcial walls; the form of the large, spatulate avicularian mandible, and the character of the spicules, all approach closely to *T. granulata*. The only difference to be observed is in the basal portion of the avicularian chamber. When completely calcified this structure becomes closed by a calcareous lamina up to the hinge denticles of the mandible, leaving only two openings similar in appearance to the opesiulæ of the ordinary zoœcia, except that they are much smaller and are removed from the lateral wall.

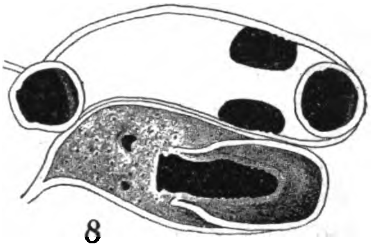


FIG. 8.—*Thalamoporella granulata* Levinsen. The variety in which the avicularium chamber is closed basally, except for two opesiulæ.

Thalamoporella falcifera (Hincks).

Hincks, 1880*b*, p. 380 (*Steganoporella rozieri*, form *falcifera*).—Levinsen, 1909, p. 186.

A single specimen of this species, which is distinguished by the falcate mandible of the avicularium, was taken in shallow water growing on algæ. Apparently this species grows only on algæ, if one may judge from the comparatively small number of records. It has a wide distribution and is recorded from Campeche Bank, Yucatan; Java Sea; Geographe Bay, Australia, and from latitude 23° 30′ N., longitude 40° W.

Genus Savignyella Levinsen, 1909.

Savignyella lafontii (Audouin).

Audouin, 1826, p. 242 (*Eucratea lafontii*).—Jelly, 1889, p. 93 (synonymy under *Eucratea lafontii*).—Norman, 1909, p. 295 (*Catenaria lafontii*, later synonymy and discussion of relationships).—Waters, 1909, p. 131 (*Catenaria lafontii*, later synonymy and discussion of generic name).—Levinsen, 1909, pp. 213 (note) and 274 (*Savignyella*, new genus and reasons for change in generic name).

This widely distributed tropical species is abundant about the islands from low water down to 10 fathoms on shells, piles, and sponges, in company with *Amathia*, *Bowerbankia*, etc. Oœcia with eggs in various stages of development are common. A number of colonies with oœcia containing eggs were on June 23 taken from the bottom of a skiff which had been in the water only since May 1. The color in life is dark brick-red, which makes the colonies quite conspicuous against the background in spite of their small size. The presence of this species in American waters has hitherto escaped notice.

While there has never been any question as to the validity of this species, there has been great confusion as to its generic position ever since its discovery. This has been sufficiently discussed by Norman (l. c.), Waters (l. c.), and Levinsen (l. c.). Waters remarks "though it certainly never ought to have been placed under *Catenaria*, it seems best to leave it here," but Levinsen solves the problem by the erection of a new genus *Savignyella*. This seems the only proper course, since the species does not have generic relationship with any of the older genera (*Catenaria*, *Eucratea*, *Alysidium*, *Catenicella*) in which it has been placed by previous authors.

GENUS HIPPOTHOA Lamouroux, 1821.

Hippothoa distans MacGillivray.

MACGILLIVRAY, 1868, p. 130.—JELLY, 1889, p. 112 (*Hippothoa flagellum*), references.—WATERS, 1904, p. 54, synonymy and references.

This minute species occurs abundantly at the Tortugas from low water to 12 fathoms, spreading over the inside of dead shells. Oœcia are abundantly developed. The "short processes arising from the side of the zoœcium, below the lateral tubular branches," described and figured by Waters (l. c.) as occurring on specimens from Sydney, New South Wales, are developed on many of the zoœcia. I am unable to add anything to Waters's description of this organ, except that it is constricted at the base where it is attached by a movable joint to the zoœcium.

The species is very widely distributed in European seas, New Zealand, Australia, Pacific Ocean, Cape Horn, etc., but has not hitherto been noticed on the American side of the Atlantic.

GENUS TRYPOSTEGA Levinsen, 1909.

Trypostega venusta (Norman).

NORMAN, 1864, p. 84 (*Lepralia venusta*) and 1909, p. 299.—GABB and HORN, 1862, p. 127 (*Lepralia inornata*).—SMITT, 1873, p. 37 (*Gemellipora glabra, forma striatula*) and p. 61 (*Lepralia inornata* Gabb and Horn).—JELLY, 1889, p. 233 (*Schizoporella striatula*), p. 237 (*S. venusta*) and p. 128 (*Lepralia inornata*).—LEVINSEN, 1902, p. 23, and 1909, p. 281 (*Trypostega venusta*).

On shells, *Steganoporella* and *Oculina*, at 5 to 15 fathoms. Smitt recorded it as *Gemellipora glabra forma striatula*, incrusting nullipores at a depth of 68 fathoms, one colony, and as *Lepralia inornata*, two colonies from 26 and 60 fathoms. After careful study of the material at hand, I am unable to distinguish more than one species, or to separate it from the *venusta* of Norman, even as a variety. The form of the aperture, the umbonate knob below, the puncturing, the character of the dwarf zoœcia and their relation to the oœcia, all appear to me to be identical. Levinsen (see above) has recently made this species the type of a new genus, *Tryposiega*, characterized especially by having the oœcia covered by dwarf zoœcia with scattered pores and a minute orifice.

This species is widely distributed, but on the American coast is known only from the Florida region. Levinsen (p. 281) included Gabb and Horn's and Smitt's records for *Lepralia inornata*, but failed to include Smitt's *Gemellipora glabra forma striatula* as a synonym. Smitt (pp. 61–62) separated the species on the following ground: "The most characteristic difference, which has caused us to place them in different families, depends on the shape of the zoœcial aperture," and his figures show some differences, but these may be accounted for by variation. In a single colony in my possession the variation is as great as that shown by Smitt's two specimens.

GENUS ADEONA Busk, 1884.

Adeona violacea (Johnston).

JOHNSTON, 1847, p. 325 (*Lepralia violacea*).—(?) REUSS, 1847, p. 85 (*Cellepora heckeli*).—SMITT, 1873, p. 30 (*Porina violacea* and *Porina plagiopora* Busk).—JELLY, 1889, p. 184 (*Microporella heckeli*), synonymy.—VERRILL, 1901, p. 54 (*Porina plagiopora*).—NORMAN, 1909, p. 296 (*Reptadeonella violacea*).—LEVINSEN, 1909, p. 283, discusses genus and species.

Taken from 5 to 18 fathoms. Both the typical form and the nominal variety *plagiopora* (Busk) occur and in some colonies the avicularia are intermediate in position between that of *violacea* and *plagiopora*. Occasionally the characters of both "varieties," together with intermediate conditions, may be observed in the same colony. The usual color is purple, varying to nearly black, or through pale bluish to white.

Smitt recorded the species "from the depth of 35 fathoms W. off Tortugas" (*Porina violacea*), and "at a depth of 60 fathoms W. off Tortugas" (*Porina plagiopora*), and Verrill has noted the form *plagiopora* at Bermuda. Otherwise the species has not been recorded from American waters.

Levinsen (1909, p. 282) has returned to the use of Busk's family Adeonidæ, thus again breaking up the arrangement established by Hincks in his family Microporellidæ. The latter family was based on the presence of a frontal pore (ascopore) as the principal character; but, as Levinsen has shown, this brought together a number of forms which are separated by more fundamental characters.

GENUS BRACEBRIDGIA MacGillivary, 1886.

Bracebridgia subsulcata (Smitt).

SMITT, 1873, p. 28 (*Porina subsulcata*).—VERRILL, 1901, p. 54 (*Porina subsulcata*).—HINCKS, 1880*a*, p. 76 (*Microporella subsulcata*).

Abundant from 10 to 12 fathoms. Branching colonies reach a height of 2 inches or more. The color in life varies from yellowish pink to orange; when dead, white. Smitt recorded the species from 10 to 48 fathoms and a dead specimen from 471 fathoms. Verrill has recorded it from Bermuda, but otherwise it is known only from the Florida region.

I have placed this species in the genus *Bracebridgia* with some hesitation, since, as this genus is understood, it possesses no ascopore (Levinsen, 1909, pp. 283, 289). In *subsulcata* a minute pore is usually present below the suboral avicularium; a similar pore appears above the basal avicularium when this organ is present. This latter pore was not figured nor mentioned by Smitt. I have not been able to determine positively the nature of this structure and it may be, from the fact that it is often wanting and also because of its presence in connection with the basal avicularium, that it is not an ascopore at all, or that it represents this organ in a vestigial condition.

The nature of the colony and the zoœcia with the marginal and other ribs, the form of the orifice, the presence of a line of special independent avicularia on each edge of a branch, all seem to agree with *Bracebridgia*. *B. pyriformis* (Busk) has a flattened area below the orifice where the avicularium is located in *subsulcata*, but the fact that MacGillivray found a single avicularium in this position in one colony of *B. pyriformis* is sufficient to show the relationship. Moreover, in the rare cases in which the oral avicularium is wanting in *subsulcata* there is a flattened area similar to that of *pyriformis*.

GENUS RETEPORA Smitt, 1867.

Retepora marsupiata Smitt.

SMITT, 1873, p. 67.—BUSK, 1884, p. 116 (*Retepora atlantica*).—GABB AND HORN, 1862, p. 138 (*Phidolophora labiata*).—JELLY, 1889, p. 212 (Jelly adopts Busk's name *atlantica* for this species, but Smitt's publication of the name *marsupiata* clearly has priority).

Taken on a number of occasions at 10 to 18 fathoms. The largest colony measured about an inch in height. The color in life is a delicate pink. Smitt records the bathymetrical distribution as 16 to 262 fathoms.

GENUS RHYNCHOZOON Hincks, 1891.

Rhynchozoon tuberculatum n. sp. (Fig. 9.)

Zoœcia small, rather evenly swollen, separated by slightly raised marginal walls; the surface, except in young individuals, strongly tuberculate. Pores are wanting, except for occasional very minute ones at the margin. Orifice ovate, the broader distal border often somewhat straight; at one side near the proximal border a strong pointed tooth extends often more than half-way across the orifice and curves backward; on the opposite side a minute projection sometimes appears, but is often entirely absent. The peristome is thin and raised high above the primary orifice, usually erect at the sides, but flaring slightly backward behind the orifice; above the large tooth the peristome appears to bear a minute, short-elliptical avicularium, but I am not able to distinguish a mandible in my specimen.

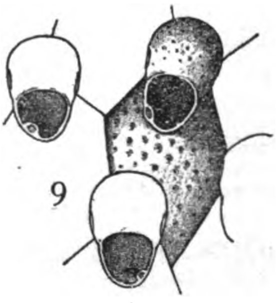
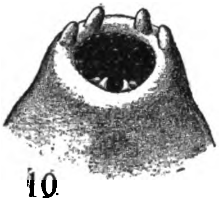


FIG. 9.—*Rhynchozoon tuberculatum* n. sp. Showing characters of zoœcium, orifice, peristome, and ovicell.

The ovicell is globose, at first smooth, but later tuberculate like the zoœcium; on either side at the widest part and near the base is a rounded thin area appearing membranous; in complete calcification the peristome rises over the front of the ovicell and fuses with the outer layer, leaving a thin, somewhat triangular area on the front.

One colony at 18 fathoms, incrusting a shell with a single layer of zoœcia.

Rhynchozoon solidum n. sp. (Figs. 10, 11, and 12.)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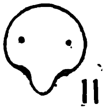
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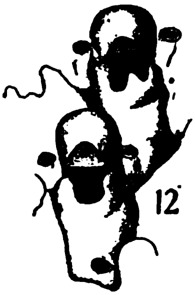
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Fig. 10.—*Rhynchozoon solidum* n. sp. Enlarged, showing primary aperture, spines, two pairs of denticles, and the beaded vestibular arch.
Fig. 11.—The same. Operculum.
Fig. 12.—The same. Fully calcified, showing avicularium, peristome, and ovicell.

Zoœcia small, little swollen, rather broad, closely set in a continuous crust, thick-walled, the surface smooth or irregularly traversed by very fine lines. As a rule only three pores are seen, one at the posterior margin and the others on either side of the orifice at the margin. Orifice evenly rounded, a little broader than long, with a rounded sinus in the proximal border. This sinus is really double, being formed by two sets of denticles, the lower pair equal in size and inclosing a semicircular sinus much like that of many *Schizoporellas.* Immediately above them is another pair of denticles, more pointed and a little longer than the lower ones, and often one of them is a trifle larger than the other; they are turned inward more than the lower pair, so as to inclose a narrower and more nearly circular sinus. The operculum is well chitinized, yellowish in color, the dots showing attachment of muscles situated nearly half-way from the circumference toward the center. Through the operculum can be seen a beaded vestibular arch very similar to that figured by Levinsen (1909, pl. XXIII, fig. 4*e*) for *R. angulatum.* In young cells there are four short, stout spines about the anterior margin of the orifice. The peristome is high and thick, extending upward and forward on the sides into thick, lappet-like processes, usually a little unsymmetrical; posteriorly the peristome extends upward and over the orifice in the form of a broad mucro, though sometimes this is but little developed.

Small avicularia, sometimes a pair, frequently only one, are placed posteriorly not far from the margin, with the triangular beak pointing laterally.

The oœcia are globose, raised, smooth, without pores; a thin extension of the peristome runs upon the top and fuses with the outer layer of the ovicell, leaving a thinner area upon the front.

One small colony incrusting a shell at 8 fathoms.

ARBORELLA, NEW GENUS.

Zoarium erect, dichotomously branched, with flexible corneous joints at the bifurcations. Zoœcia arranged in pairs, back to back, each pair facing at right angles to the preceding pair of the series, giving a somewhat quadrangular appearance. Front wall porous, but without special enlarged pores. Fertile zoœcia shorter than the infertile. Orifice with a distinct sinus. Ovicell external, but inserted partly under the front of the zoœcium, its orifice closed by the zoœcial operculum. Spines and avicularia absent. A clear, chitinous, uncalcified ectocyst covers the zoœcium and ovicell.

The relationships of this genus can not as yet be stated positively. The general appearance of the colony and especially the character of the joints and the membranous ectocyst seem to relate it to *Tubucellaria*, but the character of the oœcium and the manner of origin of the zoœcia at the tips of the branches, together with the absence of a special median frontal pore, distinctly separate it from this genus. It may be necessary to erect a new family to accommodate it. Sectioned material will be studied to determine the points necessary for a more complete understanding of the relationships.

Arborella dichotoma n. sp. (Figs. 13 to 15.)

Zoarium erect, dichotomously branching, with hinged, flexible joints at the points of bifurcation; internodes consisting of 8 to 18 pairs of zoœcia, each pair set slightly in advance and at right angles to the preceding pair of the series, facing in four directions. Zoœcia broad fusiform, wedge-shaped at the base where they are inserted between the preceding pair. The front wall evenly arched, cryptocyst well calcified, porous, with no specialized, larger pores. Ectocyst uncalcified and covering the calcareous wall as a clear layer. The orifice is evenly rounded in front and on the sides, somewhat straighter on the posterior margin, with a broad, shallow, but very evident sinus. The operculum is compound, well chitinized, with an arched rib on either side, extending inward and upward from the hinge denticle. Avicularia and spines are wanting. The fertile zoœcia are somewhat different in form from the ordinary ones, being shorter and somewhat broader, and the oœcium incloses nearly all of the orifice. The oœcium is rounded in outline, large and prominent, the aperture looking upward and closed by the zoœcial operculum. The oœcial wall is porous like that of the zoœcium and is covered by the clear ectocyst.

The colony was evidently broken off in dredging and no description can be given of the base or the mode of attachment.

One colony 0.5 inch high, composed of 24 internodes, taken at 10 fathoms on a sponge bed between Loggerhead and Garden Keys, Tortugas.

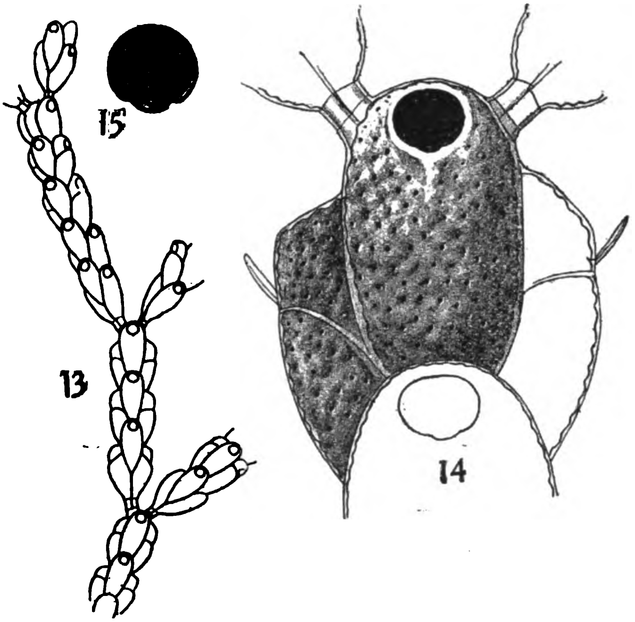


FIG. 13.—*Arborella dichotoma* n. sp. Portion of colony, showing mode of growth.
FIG. 14.—The same, showing details.
FIG. 15.—The same, operculum.

GENUS TUBUCELLARIA d'Orbigny, 1850–52.

Tubucellaria cereoides (Solander).

SOLANDER, 1786, p. 26 (*Cellaria cereoides*).—WATERS, 1907, p. 129.—LEVINSEN, 1909, p. 305.

One small colony about an inch in height was taken at a depth of 15 fathoms. This species has not before been taken on the American side of the Atlantic.

Waters makes *T. opuntioides* Pallas a synonym of *cereoides*, but Levinsen believes that they should be kept separate. This difference of opinion, which has existed since the discovery of the form, does not seem likely to reach a settlement soon. The single specimen taken by me at the Tortugas is not sufficient to decide the point. It agrees closely with specimens of *cereoides* of the same size taken at Naples, but *cereoides* has hitherto been limited to the Mediterranean by those who separate the species, while Atlantic specimens are considered to be *opuntioides*.

GENUS ESCHARELLA (PART) Gray, 1848.

Escharella costifera n. sp. (Fig. 16.)

Zoœcia of moderate size, rather regularly ovoid in form, evenly swollen, nearly hyaline. Around the margin is a row of rather large pores, between which rise strong, conspicuous ribs which converge regularly toward the central portion of the front wall. They fade out, however, before they reach it, leaving a smooth central area devoid of ribs. The orifice, which is placed far forward, is rounded in front, more straight on the posterior border, and the hinge denticles are rather conspicuous. A few zoœcia seem to show a slight median denticle, but I can not be certain of this. The peristome, which is very thin, rises in front and on the sides into a short tube, and is beset with six or eight long spines which are jointed at intervals. Posteriorly the peristome rises into a high, pointed mucro which projects forward over the orifice. This mucro is flat and thin, with a strong rib on each edge, looking as though it originated from two spines fusing at the tip, leaving an open space between. The development of the mucro, however, shows that this is not the case. The thin area forming the middle of the mucro is rounded below and pointed above and looks as though it were meant to harbor an avicularium, but no such structure is present.

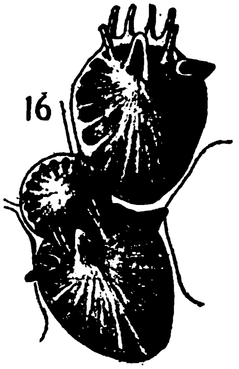


Fig. 16.—***Escharella costifera*** **n. sp. Details of front wall, mucro, avicularium, jointed spines, vestibular arch (in upper figure), and ovicell (in lower figure).**

Avicularia are present on the zoœcium, usually situated near the margin on either side of the mucronate process. Occasionally only one is present. The avicularium is comparatively large, the mandible long triangular, projecting sidewise or a trifle backward and pointing strongly upward, The oœcium is rounded in outline, prominent, imperforate, smooth on the upper surface, or with fine, radiating lines. Around the margin is a strong, raised rib, within which there is a row of large pores with strong ribs running upward as on the zoœcial wall. The anterior pair of spines is inclosed within the ovicell.

Two small colonies, the largest scarcely over 0.125 inch across, incrusting algæ, at 2 fathoms. In spite of the small size nearly every cell is adult, with a complete oœcium.

I am a little uncertain as to the generic position of this species. *Escharella* as amended by Levinsen (1909, p. 315) includes only forms without avicularia. In spite of this difference the species seems to fit better into *Escharella* than into any related genus. The absence of zoœcial and oœcial pores, except the marginal ones, the well-developed vestibular arch, the very thin operculum, the character of the orifice and peristome, and the nature of the jointed spines, seem to locate the species in *Escharella*. In many respects it resembles *E. diaphana* MacGillivray, as figured by Levinsen (l. c.), except for the presence of the avicularia and the strong development of the ribs, which are wanting in *diaphana*.

Genus Schizoporella Hincks, 1880.

Schizoporella unicornis (Johnston).

Johnston, 1847, p. 320 (*Lepralia unicornis*).—Desor, 1848, p. 66 (*Lepralia variolosa*).—Leidy, 1855, p. 10 (*Escharina variabilis*).—Smitt, 1873, p. 44 (*Hippothoa isabelleana*).—Verrill and Smith, 1873, p. 713 (*Escharella variabilis*).—Verrill, 1875*b*, p. 41 (*Hippothoa variabilis*); ibid., p. 41, pl. III, fig. 1 (*Hippothoa reversa*, n. sp.); 1878, p. 305 (*H. variabilis*); 1879*b*, p. 193, and 1880, p. 30 (*Escharina isabelliana* d'Orbigny, *E. reversa* Verrill and *E. ansata* Gray); 1900, p. 592 (? *Schizoporella isabelliana*).—Levinsen, 1909, p. 323.—Osburn, 1912, p. 236.

Abundant from low water to 10 fathoms, in the drift and growing profusely on the piles of docks, incrusting shells, and coral rocks, and rising into branched, finger-like processes about worm-tubes and the stems of hydroids, or occasionally growing free. Masses nearly as large as a man's head were dredged. The color varies from white or pale pink or purple in young, rapidly growing colonies, to a dark purplish red or sometimes nearly black. Colonies 1.5 inches in diameter were found incrusting the bottom of a skiff that had been in the water only from May 1 to June 23.

Smitt recorded the incrusting form "growing in a single layer of whitish and yellowish hue on *Oculina* and *Nullipora* at the depth of 42 fathoms," and, "a more compound colony, composed of several concentric layers of a purplish-blue tint, around an axis of foreign matter," at a depth of 17 fathoms.

Schizoporella floridana n. sp. (Figs. 17 and 18.)

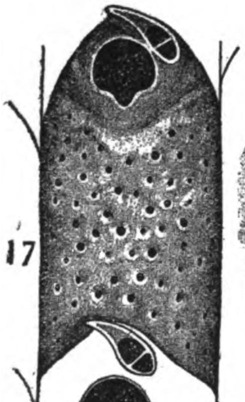
Primary characters of the zoœcium much like those of *S. unicornis* (Johnston) but with a slightly more V-shaped sinus; avicularia situated far forward, often in advance of the middle of the orifice, and with the mandible usually strongly curved toward the zoœcial axis; large vicarious avicularia with long-pointed, straight mandibles on hemispherical cells raised high above the surface of the colony.

The naked-eye appearance of the colonies is very similar to that of *S. unicornis* (Johnston) and the general characters of the zoœcia are not very different from that species. It does differ remarkably, however, in the presence of the large, vicarious avicularia, which are sometimes very abundant. These are raised high above the surface of the colony as rounded, knob-like prominences; the mandibles, while broad at the base, are very much narrowed and elongated distally. These are turned in all directions on the colony without any apparent arrangement. *Schizoporella ampla* Kirkpatrick, from Mauritius, and *S. viridis* Thornley, from the Red and Mediterranean Seas, also have vicarious avicularia.

The ordinary avicularia are situated at the side of the orifice and the mandibles are curved toward the axis of the zoœcium so as to nearly conform to the curve of the margin of the orifice. There is much variation in this avicularium and the mandible is occasionally straight instead of curved, and it may be directed backward or sideways. It is usually situated well forward near the anterior border of the orifice, but may be placed farther back, occasionally behind the orifice. The zoœcia are swollen about as in *unicornis*, the punctures are similar to that species, and the form of the orifice (with the sinus) might readily pass for that species. No oral spines have been observed. In the very young stage a low, raised margin separates the cells, but this is soon obscured by the secondary calcification. An umbo is developed below the orifice, apparently only by secondary calcification. In young colonies, where the vicarious avicularia are not yet developed, this species may readily be mistaken for *S. unicornis*, but the more anterior position and the curved form of the avicularia will serve to distinguish it even in this condition.

The oœcium differs from that of any *Schizoporella* with which I am acquainted. It is comparatively small, very short (about two-thirds as long as wide), very high and prominent, smooth, and imperforate.

This well-marked species, which appears to be undescribed, was dredged in 15 to 18 fathoms, incrusting shells and hard sponges, and, in one case, forming an erect nodular growth nearly an inch in height and about half an inch in diameter around some foreign matter as a center. The color ranges from pure white to dark purplish-red.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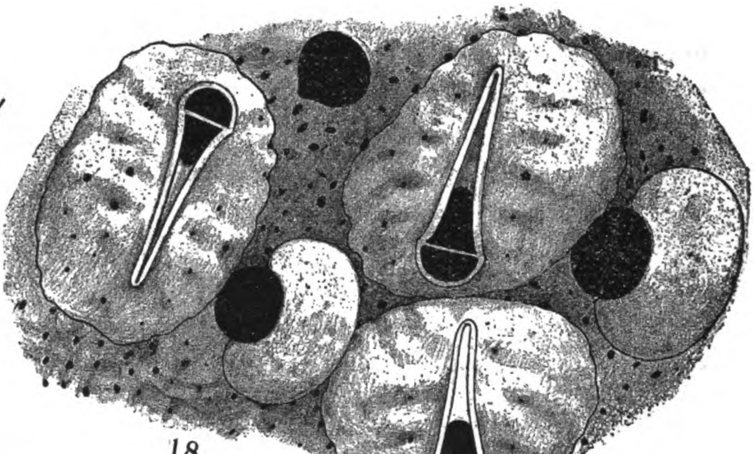
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FIG. 17.—*Schisoporella floridana* n. sp. Details of young zoœcium.
FIG. 18.—The same. Highly calcified condition, showing ovicells and the long, independent avicularia mounted on large, swollen cells.

Schizoporella sanguinea (Norman).

NORMAN, 1868, p. 222 (*Hemeschara sanguinea*).—JELLY, 1889, p. 233, synonymy.

Taken at 15 fathoms on a *Vermetus* shell; one colony with ovicells.

Reported by Smitt from Pourtales's collections at 60 fathoms, southwest of Tortugas.

Schizoporella biaperta (Michelin).

MICHELIN, 1841–2, p. 330 (*Eschara biaperta*).—SMITT, 1873, p. 46 (*Hippothoa biaperta*) and p. 47 (*Hippothoa divergens* n. sp.).—VERRILL, 1875*b*, p. 41 (*Hippothoa biaperta*); 1878, p. 305, 1879*b*, p. 30, and 1880, p. 193 (*Escharina biaperta*).—HINCKS, 1880, p. 258 (*S. biaperta* var. *divergens*).—NORMAN, 1909, p. 303 (*S. biaperta* var. *divergens*).—LEVINSEN, 1909, p. 323 (discusses and amends genus).—OSBURN, 1912, p. 237.

A few colonies of this species were taken at various depths from low water to 22 fathoms. The appearance is characteristic and the specimens compare very well with those from the Woods Hole region, but never reach such a luxuriant development.

Recorded by Smitt (under *Hippothoa biaperta*) from 9 to 60 fathoms, and (under *H. divergens* new species) from 135 fathoms "*forma typica*," and 120 fathoms "*forma laxa*."

Smitt's "*H. divergens*" has been ranked by Hincks and Norman as a variety of *biaperta*, and recorded by them from the British Isles and Madeira. All specimens examined by the writer belong to the typical *biaperta*.

Schizoporella spongites (Pallas).

PALLAS, 1766, p. 45 (*Eschara spongites*).—SMITT, 1873, p. 42 (*Hippothoa spongites*).—VERRILL, 1900, p. 592 (*Hippothoa* or *Schizoporella spongites*).—LEVINSEN, 1909, p. 324 (full discussion of the species).

One of the most characteristic species of the region; abundant from low water to 18 fathoms; incrusting shells, coral, rocks, and growing on the surface of harder sponges; also on piles. The very large oœcia serve to distinguish this species at a glance from any related forms. In life the color varies from translucent white or yellow to bright brick-red.

Recorded by Smitt from Pourtales's collections from 13 to 35 fathoms and by Verrill (l. c.) from Bermuda. Levinsen (l. c.) records it from St. Thomas and St. John, West Indies, and from Malacca (with some differences).

GENUS ESCHARINA (PART) Milne-Edwards, 1838.

Escharina pesanseris (Smitt).

SMITT, 1873, p. 42 (*Hippothoa pes anseris*).—JELLY, 1889, p. 141 (*Mastigophora dutertrei* var. *pesanseris*).—WATERS, 1909, p. 169 (*Schizoporella pesanseris*).—NORMAN, 1909, p. 302.—LEVINSEN, 1909, p. 326 (discusses genus, amended, and species fully).

One small dead specimen, composed of about a dozen zoœcia, was taken at a depth of 8 fathoms. The vibracula characteristic of the species were broken off. Oœcia present.

Smitt described this species from "two small colonies growing on a *Nullipora*, west of Tortugas" at 42 fathoms. It occurs also at Madeira in 70 fathoms (Norman), the Island of Mauritius (Kirkpatrick), the Red Sea (Waters), and Siam (Levinsen).

GENUS MICROPORELLA (PART) Hincks, 1877.

Microporella ciliata (Pallas).

PALLAS, 1766, p. 38 (*Eschara ciliata*).—SMITT, 1873, p. 26 (*Porellina ciliata*).—PACKARD, 1867, p. 270 (*Lepralia ciliata*).—VERRILL, 1879*b*, p. 29 (*Porellina ciliata*); 1875*c*, p. 53; 1880, p. 190, and 1879, p. 29 (*Porellina stellata* for a form of this species).—OSBURN, 1912, p. 233 (*M. ciliata*), and p. 234 (the variety *stellata* Verrill).—LEVINSEN, 1909, p. 328 (discusses genus).

From 5 to 18 fathoms on shells, corals, etc. One fine colony growing on an egg case of a large mollusk. Smitt records it at various depths from 7 to 60 fathoms. It is one of the most widely distributed of all bryozoa and occurs in all oceans.

Levinsen (l. c.) places this genus in family Escharellidæ.

GENUS SMITTINA NORMAN, 1903.

Smittina trispinosa (Johnston).

JOHNSTON, 1838, p. 280 (*Lepralia trispinosa*).—DAWSON, 1859, p. 256 (*Lepralia trispinosa*).—PACKARD, 1867, p. 67 (*Lepralia trispinosa*).—SMITT, 1873, p. 59 (*Escharella jacotini*), and p. 60 (*E. spathulata*).—VERRILL, 1879*b*, p. 31; 1880, p. 195 (*Mucronella jacotini*); 1875*a*, p. 514, 1878, p. 305, and 1879*b*, p. 30 (*Discopora nitida*).—WHITEAVES, 1901, p. 106 (*Smittia trispinosa*).—NORMAN, 1903, p. 120 (*Smittina* nom. nov. for *Smittia* preoccupied in Diptera).—OSBURN, 1912, p. 246 (*Smittia trispinosa*).

One of the most abundant and characteristic species in the Tortugas region from low water to 12 fathoms. A cosmopolitan species and distributed all along the Atlantic coast northward to the Arctic region. Smitt recorded it from 13 to 44 fathoms in Florida waters.

Smitt states (p. 60) "For the peculiarity of the development of the avicularia, the Floridan form, as a distinct variety, I propose to be named *Escharella spathulata*." But variation in the secondary characters of this species here as in many other regions occurs to a bewildering extent. The typical form with the large, pointed avicularia, common in northern waters, is rare, but these are occasionally observed. One beautiful colony growing on the under side of *Cupularia guiniensis* at 10 fathoms is an almost pure example of Smitt's *spathulata*. Even the smaller, triangular avicularia figured by Smitt (pl. x, fig. 200) are not represented, but in a few cases very elongated pointed avicularia occupy the place of the long-spatulate form figured by Smitt. Another colony found in the drift is more like Verrill's *nitida* in the character of the avicularia, which are small, short-pointed, and short oval in form, very much like specimens from the southern New England coast. In a few cases very large spatulate avicularia, as long as the whole zoœcium, were observed. In one colony short-triangular avicularia were rather regularly disposed in pairs below the orifice with the mandibles pointing outward. Occasionally the avicularia are almost wanting from a colony, a condition seen in many New England specimens that bear evidence of very rapid growth in a single layer.

The oœcia vary much with the state of calcification. In younger stages they are coarsely and sometimes very irregularly punctured. This layer may be entirely covered by secondary calcification, when the oœcia present a smooth, shining appearance or are variously roughened. The secondary layer usually grows irregularly over the primary layer, but in some cases it forms a rather regular raised border with a rounded area of the primary layer exposed on the top.

This form resembles closely the variety *protecta* Thornely, as figured by Waters (1909, pl. 17, fig. 5), but the large avicularia are evenly rounded at the tip instead of being divided into points. The peristome also varies greatly. In some cases it is scarcely raised; in others it takes the form of a tall, broad, median projection, often forked at the tip and projecting so far over the orifice as to nearly obscure it from above. All sorts of intermediate conditions are observed. The peristome also occasionally shows raised lappet-like projections at the side of the orifice, as seen commonly in specimens from Massachusetts (Osburn, 1912, pl. XXVII, fig. 65*a*, 66). In spite of all these variations in secondary characters there is a remarkable constancy in the primary orifice, denticle, oral spines, and the primary zoœcial walls.

Another variation, and one which I am at a loss to explain, is this: Some colonies consist of zoœcia, the secondary characters of which are all of one type or with little variation, while other colonies will show not only zoœcia representing two or more of the so-called varieties, but all sorts of intergradations as well.

GENUS LEPRALIA (PART) Johnston, 1847.

Lepralia audouinii (d'Orbigny).

D'ORBIGNY, 1850–2, p. 401 (*Cellepora audouinii*).—SMITT, 1873, p. 56 (*Escharella audouinii*).

This species, which is known from the Red and Mediterranean Seas, occurs at the Tortugas from low water to 10 fathoms. Colonies taken from the bottom of a skiff which had been in the water from May 1 to June 23 were an inch in diameter, with oœcia containing embryos. In life the colonies are translucent white, but the oœcia often appear light pink when embryos are present.

Smitt's record was based on a single colony growing on a nullipore at 37 fathoms, west of Tortugas.

Lepralia porcellana Busk.

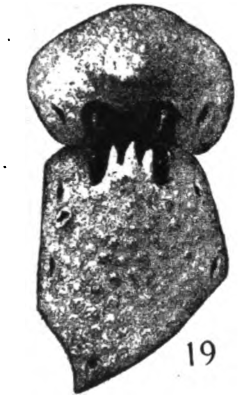
BUSK, 1860, p. 283, pl. 31, fig. 3.—SMITT, 1873, p. 62 (*Lepralia cleidostoma*).—NORMAN, 1909, p. 305, synonymy.

Taken at 5 to 15 fathoms on shells and on other bryozoa, such as *Steganoporella magnilabris* and *Holoporella turrita*. The key-hole form of the orifice, the pointed avicularia with elongate mandibles, and the absence of pores except occasional ones at the margin serve to distinguish the species readily. Oœcia present. In one case there is a vicarious avicularium, somewhat larger than the usual type, mounted on a smooth, swollen chamber. A low, smooth, umbonate process is sometimes present below the orifice.

Recorded by Smitt from Pourtales's collections from 30 to 120 fathoms.

Lepralia uvulifera n. sp. (Figs. 19 and 20.)

Zoœcia small, the surface shining and covered by small, smooth knobs. Pores are wanting except for 3 to 5 large, marginal pores. Usually these are placed as follows: One on either side near the orifice, one on either side about half-way back, and a single pore at the posterior end. The orifice is slightly broader than long, evenly rounded anteriorly, nearly straight on the posterior border, hinge teeth scarcely noticeable. Peristome slightly raised anteriorly and beset with about six slender spines. Posteriorly the peristome rises into a strong, mucronate process, which in complete calcification is divided at the tip into three sharp spines. A small avicularium is present on one zoœcium, situated somewhat behind the orifice near the lateral border, with the pointed mandible pointing backward and outward. The oœcium is comparatively large, heavily calcified, and covered with knobs like those of the zoœcium; a high umbonate process rises on the top, and there is a single large pore on either side of the oœciostome near the base; the oœcial orifice is wide and high, with a calcified projection extending nearly vertically downward into the orifice and bearing a close resemblance to the uvula of the human palate, except that it is a little more truncate at the tip.



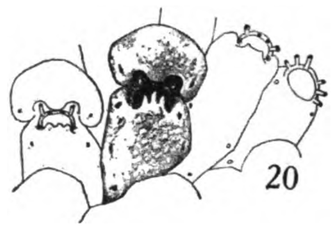


FIG. 19.—*Lepralia uvulifera*, n. sp.
FIG. 20.—The same. Portion of colony showing at the right the orifice of a young zoœcium.

One small colony of perhaps three dozen zoœcia incrusting the under side of a *Cupularia guiniensis* at 10 fathoms.

This species is certainly closely related to *L. watersi* Calvet (1906, p. 412, pl. XXVII, fig. 11) from the Cape Verde Islands, but it differs in having the umbo strongly trifid instead of merely pointed; in the more open form of the ovicell, which partially incloses the anterior part of the peristome, instead of being placed in front of it as in *L. watersi;* and also in lacking the complete lower border of the oœciostome shown in the figure of *L. watersi.* Calvet's figure also shows the oral spines situated slightly outside of the oral border, while in *uvulifera* the spines are on the border.

Lepralia cucullata Busk.

Busk, 1854, p. 81.—Waters, 1909, p. 150, synonymy and references.—Jullien et Calvet, 1903, p. 141 (*Schizoporella cucullata*).

Common in shallow water at the Tortugas. In life the color of the colony is dark brownish-purple, sometimes almost black, and the polypides in the young zoœcia at the edge of the colony have a fine carmine color. A number of colonies more than an inch in diameter were found on the bottom of a skiff that had been in the water only from May 1 to June 23.

The generic position of this well-known species is very unsettled. A majority of authors have agreed in placing it in *Lepralia*, others in *Smittia*, and still others in *Schizoporella*. The reason for this difference of opinion lies in the general simplicity of the zoœcia, which lack characters usually considered as diagnostic. The form of the orifice is somewhat intermediate between that of *Lepralia* and *Schizoporella*. There is a very shallow curve on the posterior border, which may be interpreted as a sinus, as Calvet has done (l. c.), or as merely the space between the hinge denticles. In the absence of any means of settling the controversy, I place the species tentatively in *Lepralia*, in which genus it was described by Busk.

Distribution: Mediterranean, Red, and Arabian Seas; Azores Islands; Cape Verde Islands; South Africa, and Cape St. Lucas, Lower California. Not before noted on the Atlantic side of America.

Lepralia rostrigera (Smitt).

Smitt, 1873, p. 57 (*Escharella rostrigera*).—Waters, 1885, p. 298; 1887, p. 61.—Jelly, 1889, p. 126 (under *L. depressa*).

The *Escharella rostrigera* of Smitt has been made a synonym of *L. depressa* Busk by Miss Jelly (l. c.), but in the opinion of the writer it represents a distinct species. Smitt recognized a relationship between the species, but indicated that the differences are constant. The study of material taken by me at the Tortugas confirms this view. The form of the zoœcial aperture is quite different, being well rounded at the posterior margin, as figured by Smitt, and there is never more than a faint indication of a constriction of the sides. The avicularia are characteristically placed far forward, alongside of the anterior part of the orifice, or frequently entirely in front of it. Busk's figure of *L. depressa* (1854, pl. 91, figs. 3 and 4) shows no indication of pores. In *rostrigera* there are numerous small pores in the frontal wall in younger stages, and large, marginal pores are present in all stages. The larger zoœcia with the broad aperture described and figured by Smitt (pl. x, fig. 205) are occasionally present in my specimens. Smitt's specimens were taken by Pourtales in 35 and 43 fathoms. Mine were taken at 10 and 15 fathoms on coral and shells. A fine colony at 10 fathoms incrusted the base of a living *Cladocora arbuscula*.

Lepralia contracta Waters var. serrata Osburn.

Waters, 1899, p. 11.—Norman, 1909, p. 306.—Osburn, 1912, p. 242 (*Lepralia serrata*).

Several colonies incrusting shells at 5 to 18 fathoms. This variety was described by me as *Lepralia serrata* n. sp., from the Woods Hole region (1912, p. 242, pl. 26, fig. 57).

After a careful study of the Florida specimens I am of the opinion that this can be only a variety of *L. contracta* Waters, described from Madeira (1899, p. 11). Waters's description is brief and his figures small, but Norman (l. c., pl. 41, fig. 56) has refigured the species. The differences seem to be largely in the secondary calcification, which appears to be much greater in American specimens; also there are six oral spines in Madeira specimens, while usually only four are to be observed in American material. The avicularia in Florida specimens are much more abundantly developed and are frequently raised high above the front of the zoœcium on small mammillary processes. Also, the lateral oral denticles are much more prominent than they appear in the figures of Madeira specimens and are strongly bifid; however, Norman figures one such bifid denticle. In one of my specimens the spatulate avicularia are extremely elongated, fully as long as the zoœcium, and, while usually directed forward, are sometimes reversed in position. The zoœcial wall appears to be much more thickened than in the Madeira specimens, to judge by the figures of Waters and Norman. Notwithstanding these differences I believe they are closely related. The form of the oœcium with the peculiar membranous area and the serrated character of the primary zoœcial orifice are identical and are so unique that I believe them to indicate the same species. Specimens from southern New England are much more highly calcified than Florida specimens, which seem in some respects to be intermediate between those from New England and Madeira.

Lepralia edax (Busk).

BUSK, 1859, p. 59 (*Cellepora edax*).—SMITT, 1873, p. 63 (*L. edax forma typica* and *calcarea*).—HINCKS, 1880, p. 311.—VERRILL, 1909, p. 54.

One colony taken at 18 fathoms, forming a cylindrical mass more than 0.25 inch in diameter and many layers in thickness, which originally surrounded a branched structure of some sort, and one colony taken at 8 fathoms incrusting a shell fragment. Smitt's figures and description of this species are very complete. I have compared these specimens with one from the English Channel sent me by Dr. S. F. Harmer. English specimens are said to be found only on certain species of gastropod shells. While Florida specimens have not been taken in such situations, I can distinguish no differences of any importance. My Tortugas specimens belong to what Smitt calls the *forma calcarea*, but it can scarcely be considered a distinct variety.

The large, pointed, vicarious avicularia described and figured by Smitt are abundantly distributed over the colony. The ovicell has a peculiar membranous area on the upper surface, a feature also possessed by *L. contracta* Waters, though in the latter species this area is much nearer the zoœcial aperture.

Smitt records the species from Pourtales's collections from 49 to 79 fathoms. Verrill records it for the Bermudas in shallow water.

Lepralia janthina (Smitt).

SMITT, 1873, p. 63 (*Lepralia edax forma janthina*).

This, I believe, must be separated from *edax* as a distinct species. The form of the orifice, a character to which Smitt gave undue importance, is similar, but in *janthina* the orifice is not only much larger, but relatively wider and shorter, with less space behind the hinge denticles. The avicularia are all pointed and are situated on a prominence behind the orifice with the mandible turned forward and frequently slightly outward. There are no vicarious avicularia. The color is a deep blue-black or violet, entirely different from any specimens of *L. edax* I have seen. The zoœcia are considerably larger than those of *L. edax*. Ovicells are wanting.

One colony 0.25 inch in diameter taken at 6 fathoms incrusting a shell. Smitt records one specimen from 13 fathoms on coral.

The *Cellepora janthina* of Waters (1899, p. 14) from Madeira can not be the *L. edax* var. *janthina* of Smitt, as Norman (1909, p. 311) points out in renaming Waters's species *C. rotundora*.

GENUS PHYLACTELLA Hincks, 1880.

Phylactella labrosa (Busk).

BUSK, 1854, p. 92 (*Lepralia labrosa*).—JELLY, 1889, p. 204.—NORMAN, 1909, p. 308.

Dredged at 22 fathoms on shells and hard sponges. Ovicells are lacking. There is no median denticle on the proximal margin of the orifice, nor are the zoœcia disposed in radiating lines, but form a solid crust. However, Norman (l. c.) has shown that both of these characters are variable.

Phylactella collaris (Norman) var. aviculifera nov. (Fig. 21.)

NORMAN, 1866, p. 204 (*Lepralia collaris*); 1909, p. 309.—JELLY, 1889, p. 203.—ROBERTSON, 1908, p. 307.

This species has not heretofore been recorded from the Atlantic side of North America, though Miss Robertson records it from southern California, and it is found on the European side of the Atlantic Ocean as far south as the Madeira Islands.

At the Tortugas, growing on shells at from 1 to 15 fathoms, occurs the variety which I have here called *aviculifera*. In all respects this appears to agree fully with *P. collaris*, except that there is a rather large avicularium situated within the collar immediately below the orifice, with a triangular mandible pointing upward, or very rarely sidewise. This appears on every zoœcium of the colonies studied. Norman has described a rounded avicu-

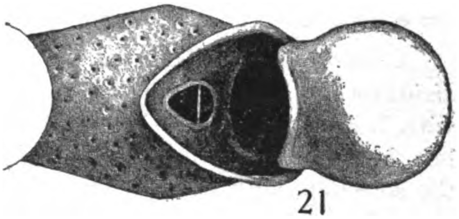


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FIG. 21.—*Phylactella collaris* (Norman) *aviculifera*, var. nov. Viewed partly from in front and showing avicularium.

larium on the lower lip of occasional zoœcia of *L. labrosa* from Madeira (1909, p. 309).

GENUS CELLEPORA Linné, 1767.

Cellepora dichotoma Hincks.

HINCKS, 1862, p. 304.—SMITT, 1873, p. 53, pl. IX, fig. 193–198 (*C. avicularis*).—JELLY, 1889, p. 51, gives synonymy and also, p. 46, includes Smitt's references in *C. avicularis* Hincks.—(?) VERRILL, 1878, p. 305 (*C. avicularis*); (?) 1901, p. 54 (*C. avicularis*).

Two well-developed specimens, 0.5 inch or more in height, taken at a depth of 10 fathoms; one was attached to a hydroid stem. There can be no doubt that these specimens belong to *C. dichotoma* instead of *C. avicularis*, as I have compared them with authenticated material from England. Smitt's figures, which he refers to *C. avicularis*, undoubtedly are *C. dichotoma*, as Hincks (1880, p. 404) has already pointed out. Smitt indicates the bathymetrical distribution as from 9 to 111 fathoms.

Verrill's records for *C. avicularis* at Fort Macon, North Carolina (1878), and Bermuda (1901) in all probability apply to *dichotoma*, though it is possible that the former should be *C. americana* Osburn (1912, p. 238), which Verrill at another time (1879B) listed as *avicularis*. *C. americana* and *C. dichotoma* have been taken at Beaufort, North Carolina, by the writer.

Cellepora verruculata Smitt.

SMITT, 1873, p. 50.—BUSK, 1884, p. 150 (*Escharoides verruculata*).—JELLY, 1889, p. 60.—CALVET, 1906, p. 444.

A number of colonies taken on shells and in similar situations from low water to 15 fathoms. Smitt described the species from a single specimen taken by Pourtales west of the Tortugas in 42 fathoms. Recorded by Busk (l. c.) off Heard Island at 75 fathoms, and by Calvet (l. c.) for the Mediterranean Sea, "Naples, côtes de Corse, Cette."

The zoœcia undergo a remarkable change in appearance with advancing calcification.

GENUS LAGENIPORA Hincks, 1877.

Lagenipora ignota Norman.

NORMAN, 1909, p. 309, pl. 42, figs. 10–13.

A colony taken at Tortugas in 12 fathoms on a dead colony of *Mucronella bisinuata* and another at 10 fathoms on a *Vermetus* shell seem to be identical with Norman's species. They differ in not rising into rounded branches, but the small size of the colonies easily accounts for this. The character of the zoœcium, with the pair of oral avicularia, is identical, and the oœcia also. The large spatulate avicularia, said by Norman to be present in extraordinary numbers, are but sparsely represented.

Hitherto known only from the Madeira Islands, where it was taken at 70 fathoms (Norman).

GENUS HOLOPORELLA Waters, 1909.

Holoporella albirostris (Smitt).

SMITT, 1873, p. 70 (*Discopora albirostris*).—BUSK, 1881, p. 346 (*Cellepora albirostris*).—JELLY, 1889, p. 45 (references under *Cellepora albirostris*).—WATERS, 1885, p. 304; 1887, p. 68 (*Cellepora albirostris*); 1909, pp. 159–161 (*Holoporella* gen. nov.).

This species, which is very characteristic of the region, occurs in abundance from low water to 15 fathoms. It grows on coral-rocks, corals, shells, and on the firmer sponges. Recorded by Smitt from Florida waters in 25 to 35 fathoms. It is readily distinguished from other species of this region by the presence of a towering, pointed white spine situated at one side of the proximal margin of the orifice. Except in the youngest stages the colony has a dark grayish or blackish color, against which the white spines stand out in sharp contrast. A very small avicularium is situated at the base of the spine, large spatulate avicularia are distributed over the colony, and vestigial, minute avicularia are occasionally present at the side of the orifice.

Waters in 1909 (l. c.) established the genus *Holoporella* to include, for the most part, those species of the old genus *Cellepora* in which the sinus is lacking and which have a rounded or straight posterior margin of the orifice, and in which also the opercular muscles are attached near the border. Levinsen (1909, p. 347) erected a new family, Holoporellidæ, to include this genus, thus farther removing it from *Cellepora*. This separation is based on the fact that in *Holoporella* the operculum is attached, sometimes by hinge teeth, to the lateral margins of the orifice, while in *Cellepora* the operculum is suspended at the edges of the sinus. Oral spines are often present in *Holoporella*, but are wanting in *Cellepora*. Rostra, with avicularia, may be present in both genera. Waters includes the following American species of the Florida region in *Holoporella: H.* (*Discopora*) *albirostris* (Smitt), *H.* (*Lepralia*) *turrita* (Smitt), and *H.* (*Discopora*) *pertusa* (Smitt). Levinsen adds *H.* (*Discopora*) *advena* (Smitt), which apparently completes the list of the described species of this region.

Holoporella pusilla (Smitt).

SMITT, 1873, p. 70 (*Discopora albirostris forma pusilla*).—BUSK, 1884, p. 436 (*Cellepora pusilla*).

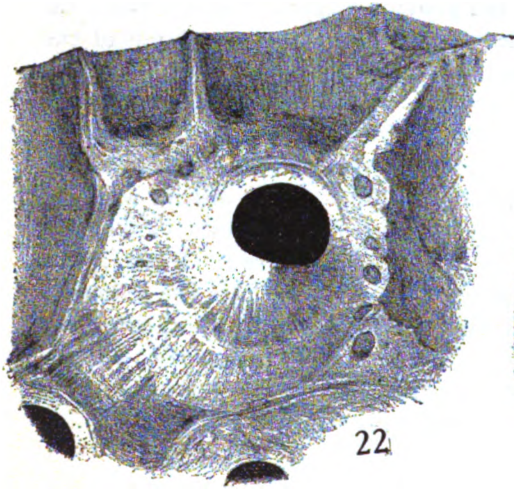
In the "Floridan Bryozoa" Smitt describes this species as a form of his *Discopora albirostris*, stating that their relationship is "incontestably proved by the very same form of the zoœcial aperture." Busk (l. c.) objects to placing *pusilla* as merely a variety of *albirostris*, giving it as his opinion that the two seem to him to be quite distinct. Although Busk possessed no material of *pusilla*, he was evidently correct in forming this opinion. If the single specimen taken by me at the Tortugas is identical, as I believe it to be, with Smitt's *pusilla*, it differs from *albirostris* in the entire absence of dark pigment; in the smaller size of the colony, the individual, and the orifice; in the presence of radiating lines of depressions and small wart-like prominences; in the smaller size of the oœcia; in the much slighter development of the rostrum, which never rises much above the avicularium; and in the more procumbent position of the rostrum, which projects forward over the orifice. The form of the orifice is, as stated by Smitt, very similar to that of *albirostris*, but (as Busk points out) this form is common to a number of species. Small hinge denticles are sometimes present. Smitt figures the species as having from 3 to 6 oral spines. There is scarcely any indication of these in the specimen in my possession. The oral avicularium is usually rounded, but occasionally the mandible is obtusely triangular. No other avicularia are present.

One colony 0.25 inch in diameter incrusting a shell at low tide.

Smitt records it from Pourtales's collections at 9 to 60 fathoms.

Holoporella magnifica n. sp. (Figs. 22 and 23.)

A very coarse species with very large zoœcia and orifices large enough to be distinguished readily by the naked eye. Taken at 10 fathoms incrusting a sponge and a very small colony on a dead shell at the same depth.



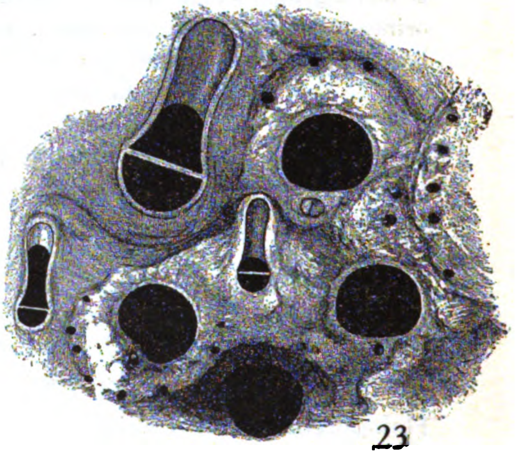


FIG. 22.—*Holoporella magnifica* n. sp. Young zoœcium at edge of colony, showing manner of growth. Rostrum and rostral avicularium not yet developed.

FIG. 23.—The same. Highly calcified portion near center of colony, showing one deeply immersed and three erect superficial zoœcia, independent avicularia of two sizes, and the rostral avicularium.

Zoœcia large, procumbent at the growing edge, erect and irregular at the center of the colony. Imperforate except around the edge, where there are large pores, often coarsely cancellated between the cells in the middle of the colony. Very heavily calcified, even in young cells. The orifice is much as in *H. albirostris*, except that it is much larger. The peristome is very thick and broad, slightly raised all around the orifice (when oœcia are present it extends over them in later stages of growth). The rostrum is small and low, projecting so slightly over the orifice as to scarcely hide the posterior margin in any case. On one side of the rostrum is placed a large avicularium with an elliptical mandible, black in color. Sometimes the rostrum is so slightly developed that the avicularium appears to be placed transversely behind the orifice. The operculum is heavily pigmented with dark brown, especially at the edge. Large, spatulate avicularia with brown or blackish mandibles are abundantly distributed over the colony. There is a slight amount of the brownish pigment in the zoœcial wall. In all the avicularia the pigment is confined to the mandible, which is thus strikingly contrasted with the colorless basal membrane. In the operculum also the color is limited strictly to the hinged portion and does not extend upon the base. The oœcia are, as in other species of this genus, shallow and widely open, and they are deeply immersed in the crust, except in the very young stage.

In addition to the specimens mentioned above, a large flat specimen, 2 inches across and many layers in thickness, which was apparently growing unattached, was taken by Dr. Paul Bartsch off Biscayne Key, Florida. It has not been possible to identify these specimens with any known species. The large size of the zoœcia and the orifice, the great thickness of the walls, even in young specimens, the straight proximal border of the orifice, the absence of a tall rostrum, and the large size of the oral avicularium, together with the elongate form of the mandible with its round tip in the vicarious avicularia, form a set of characters which serve to distinguish it.

Holoporella turrita (Smitt).

SMITT, 1873, p. 65 (*Lepralia turrita*).—WATERS, 1883, p. 438 (*Smittia turrita*); 1909 p. 161 (includes in *Holoporella*, new genus).—RIDLEY, 1881, p. 55 (*Cellepora turrita*).—JELLY, 1889, p. 253 (*Smittia turrita*).

A very common species at 12 to 15 fathoms, growing on shells, corals, and the firmer sponges. Recorded by Smitt from Pourtales's collections as not frequent, only a few specimens taken, at from 26 to 44 fathoms, growing on corals and nullipores.

Color in life bright pink to brick-red. Above the spreading incrustations rise knob-like projections, usually 0.25 inch or so in diameter, sometimes much larger. The younger zoœcia are separated by delicate, raised, white walls which are very conspicuous against the red color of the colony. The white points of the blunt spines are also strongly contrasted with the ground color.

GENUS PETRALIA MacGillivray, 1869.

Petralia bisinuata (Smitt).

SMITT, 1873, p. 59 (*Escharella bisinuata*).—LEVINSEN, 1909, pp. 350–51.

This well-marked species, which has not been noted outside of the Florida region, is common at the Tortugas on shells and sponges, from 10 to 18 fathoms. The color in life is bright vermilion. Smitt described the species from Pourtales's collections at 9 and 19 fathoms.

Levinsen (see above) has placed this species in MacGillivray's genus *Petralia* and has erected a new family, Petraliidæ, to include this genus.

CTENOSTOMATA.

GENUS BOWERBANKIA Farre, 1837.

Bowerbankia gracilis Leidy.

LEIDY, 1855, p. 142.—HINCKS, 1877, p. 215 (*Valkeria caudata*); 1880, p. 521 (*Bowerbankia caudata*).—VERRILL AND SMITH, 1873, p. 709 (*Vesicularia gracilis*), and p. 710 (*V. fusca*).—VERRILL, 1880, p. 28 (*Vesicularia gracilis*).—LEVINSEN, 1894, p. 82 (*Bowerbankia caudata*).—OSBURN, 1912, pp. 253–245 (synonymy); 1912*a*, p. 287.

Specimens of this species, which do not seem to differ from Woods Hole material, were taken in the moat at Fort Jefferson and on the piles of the docks, attached to shells, or creeping over the sea-weed, and covered to such an extent by coral mud as to almost obscure them. No erect branches were noted. Many individuals show the caudate process of the variety *caudata* (Hincks).

On the North American coast the species has been identified by the writer from Nova Scotia, Maine, Massachusetts, Connecticut, New Jersey, North Carolina, and Florida. Otherwise it is known from England and Denmark.

It is quite probable that the genus *Bowerbankia*, as Waters (1910, pp. 241 and 244) suggests, must be fused with *Zoobotryon*, but as Waters himself keeps them separate pending more thorough studies of the ctenostomes, the writer has not the temerity to combine them. The only marked distinguishing characters are the much greater softness and hyalinity of the stock and the absence of a creeping stolon in *Zoobotryon*. These characters will readily separate the one species of *Zoobotryon* from any *Bowerbankia* with which the writer is acquainted.

GENUS ZOOBOTRYON Ehrenberg, 1831.

Zoobotryon pellucidum Ehrenberg.

EHRENBERG, 1831, pl. III, fig. 10.—SONDER in Coll. Binder (*Ascothamnion trinitatis*).—WATERS, 1910, p. 243.

This species, first described from the Mediterranean, is common at the Tortugas, floating and attached, especially in the Fort Jefferson moat and about the piles of the dock. One colony 8 inches across was observed. The zoœcia in all cases were much obscured by a layer of white coral mud adhering to the ectocyst.

Not previously recorded for the Florida region. Sonder (see above) recorded it as a plant from the island of Trinidad, and according to Waters (see above) it is known from the Isle of Pines, South Australia, Zanzibar, the Red Sea, and the Cape Verde Islands.

GENUS CYLINDRŒCIUM Hincks, 1880.

Cylindrœcium giganteum (Busk).

BUSK, 1856, p. 93 (*Farrella gigantea*).—HINCKS, 1880, p. 535.

This species, which has been recorded from the British Isles, from various localities in the Mediterranean Sea, from the Red Sea, and from the Queen

Charlotte Islands, has not hitherto been noted on the American side of the Atlantic. It is abundant at the Tortugas from low water down to several fathoms, growing profusely over piles, shells, seaweed, and sponges. The zoœcial wall in all cases was thickly covered with a layer of coral mud. Occasional individuals showed the swelling at the side of the zoœciostome which forms the oœcium. Embryos in various stages of development were present.

GENUS ANGUINELLA van Beneden, 1844.

Anguinella palmata van Beneden.

VAN BENEDEN, 1844, p. 58.—BUSK, 1856, p. 95.—OSBURN, 1912, p. 253, pl. XXVIII, fig. 78.

This species occurs rather sparingly among algæ on the reefs in shallow water. It is very easily overlooked because its irregularly branched form gives it a marked resemblance to some of the smaller algæ. The species is now known to occur on the American coast from the Tortugas to Buzzard's Bay, Massachusetts (Osburn, 1912). At Beaufort, North Carolina, it is very abundant and grows much larger than at the localities mentioned above. It has not previously been noticed south of Charleston, South Carolina, where it was recorded by Busk (Harvey, coll.).

GENUS AMATHIA Lamouroux, 1812.

? Amathia goodei Verrill.

VERRILL, 1901, p. 329.—(?) SMITT, 1872, p. 4 (*Serialaria* sp. undet.).

Several colonies, growing attached to piles, seem to belong to this species, which Verrill described from the Bermudas. Verrill described the branches as "thick, soft and flaccid" and the zoöids as "numerous, arranged in large, dense elongated clusters composed of several close rows, which often nearly or quite surround the stem, but are scarcely at all spiral."

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DEPARTMENT OF MARINE BIOLOGY
OF
CARNEGIE INSTITUTION OF WASHINGTON

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PAPERS FROM THE TORTUGAS LABORATORY
OF THE
CARNEGIE INSTITUTION OF WASHINGTON

VOLUME VI

WASHINGTON, D. C.
PUBLISHED BY THE CARNEGIE INSTITUTION OF WASHINGTON
1914

CARNEGIE INSTITUTION OF WASHINGTON

PUBLICATION NO. 183

Copies of this Book
were first issued
JUL 14 1914

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

CONTENTS.

PAGE.
I. The Effects of Temperature upon Tropical Marine Animals. By A. G. Mayer.......8 figs., 4 records 1–24
II. The Relation between Degree of Concentration of the Electrolytes of Sea-water and Rate of Nerve-Conduction in *Cassiopea*. By A. G. Mayer.......13 figs. 25–54
III. The Law Governing the Loss of Weight in Starving *Cassiopea*. By A. G. Mayer.......1 plate, 21 figs. 55–82
IV. Changes in Salinity and their Effects upon the Regeneration of *Cassiopea xamachana*. By A. J. Goldfarb.......4 figs. 83–94
V. Regeneration in the Annelid Worm, *Amphinoma pacifica*, after Removal of the Central Nervous System. By A. J. Goldfarb....... 95–102
VI. Experimentally Fused Larvæ of Echinoderms with Special Reference to their Skeletons. By A. J. Goldfarb.......15 figs. 103–121
VII. Experiments on the Permeability of Cells. By J. F. McClendon...3 figs. 123–130
VIII. The Relation Between the Rate of Penetration of Marine Tissues by Alkali and the Change in Functional Activity Induced by the Alkali. By E. Newton Harvey.......1 fig. 131–146
IX. Physiological Studies on Certain Protozoan Parasites of *Diadema setosum*. By Merkel H. Jacobs....... 147–157
X. The Origin of the Electric Tissues of *Gymnarchus niloticus*. By Ulric Dahlgren.......9 plates, 9 text-figs. 159–194
XI. The Development of the Apyrene Spermatozoa of *Strombus bituberculatus*. By E. E. Reinke.......7 plates 195–239
XII. History of the Spotted Eagle Ray, *Aëtobatus narinari*, with a Study of its External Structures. By E. W. Gudger.......10 plates, 19 figs. 241–323

I.

THE EFFECTS OF TEMPERATURE UPON TROPICAL MARINE ANIMALS.

BY ALFRED GOLDSBOROUGH MAYER,
Director of Department of Marine Biology of the Carnegie Institution of Washington.

Eight figures and four records.

THE EFFECTS OF TEMPERATURE UPON TROPICAL MARINE ANIMALS.

By Alfred Goldsborough Mayer.

CONCLUSIONS.

Tropical marine animals commonly live within 5° C. of their temperature of maximum activity and within 10° to 15° C. of their upper death temperature. In marine animals of the temperate or arctic regions a considerable range of temperature above or below the normal produces relatively little difference in their activities, but in tropical forms even a few degrees of heat or cold cause a marked depression in movement. In tropical Scyphomedusæ this depression of movement appears to augment about as the square of the change in temperature from that of the optimum. Thus it is more depressant than one would expect were it due to a simple chemical reaction.

As was shown by Harvey (1911), the rate of nerve-conduction in the subumbrella tissue of *Cassiopea* varies as the increment of temperature, increasing in a linear ratio as the temperature is raised.

Experiments conducted at the Murray Islands, Australian Great Barrier Reef, show that those corals which die at temperatures below 36.5° are killed by being buried 11 hours under the mud, but those which resist 37°, and above, are proportionally less sensitive to the smothering effects of mud and may survive being buried for 30 to 40 hours. This suggests that high temperature produces death by causing asphyxiation, the oxygen of the sea-water being insufficient to sustain the increased metabolic activity of the animal.

OBSERVATIONS AND EXPERIMENTS.

The literature upon the effects of temperature on animal life and activities is both extensive and widely scattered, although Davenport and Castle (1895), in their paper in the "Archiv für Entwicklungsmechanik der Organismen," Bd. 2, have given a list of all previous papers treating of experiments upon the maximum temperature which can be endured by aquatic organisms; and Bachmetjew (1899), in the "Zeitschrift für wissenschaftliche Zoologie," Bd. 66, presents a complete résumé of researches upon the subject of the temperature of insects in relation to that of their surroundings. Also a review of works upon the physiological effects of temperature is given by Demoll and Strohl (1909).

Probably no single factor is a more effective barrier to the extensive geographical range of marine animals than is that of temperature. This fact has long been recognized and there are many striking examples of its general truth; for example, Professor Hjort, in Murray and Hjort's "Depths of the Ocean" (1912, p. 444), concludes that the southern limit of northern boreal species of fishes from the sea-bottom everywhere coincides with the isotherm of 10° C. at a depth of 100 meters.

That temperature should be so important a barrier to universal distribution is the more remarkable in view of the large number of observations showing how readily animals may become artificially adapted to survive in unaccustomed temperatures. Thus Davenport and Castle (1895) found that tadpoles reared at about 15° C. go into heat-rigor at 40° to 41° C., but if kept at about 24.5° C. for 4 weeks their heat-rigor temperature is raised to 43° to 43.8° C.

The experiments of Dallinger (1887) are even more remarkable, for he kept *Flagellata* in a warm atmosphere beginning at 15.6° C., gradually raising the temperature for several years until it became 70° C., the colony still surviving and reproducing normally, although in nature the same species were killed at 23° C.

In studying animals in a state of nature, Vernon (1899) found that at Naples the mean death-temperatures of various marine invertebrates ranged from 34° to 42.3° C., but that the death-temperatures of the same species in July and August are from 0.6° to 1.3° C. higher than in March and April, this change being associated with a rise of about 10° in the surface temperature of the Mediterranean. Observations of similar purport were made by King (1903) upon the effects of seasonal temperature on the development of toad eggs.

It is, therefore, somewhat surprising, when we see how readily certain animals may become acclimated in a short time to abnormally high or low temperatures, that whole races of marine organisms are confined within a narrow range of temperature. For example, the rhizostomous scyphomedusæ are restricted to tropical or warm seas, none of them extending into regions beyond the summer isotherm of 16° C. Also, the species of the genus *Cyanea* are equally well confined to cold seas, being bounded in their range from either pole by the summer isotherm of 20°. On the other hand, *Aurellia aurita* is eurythermal, ranging from pole to pole and giving rise to many varieties and local races.

Among the 440 known species of hydromedusæ only one, *Solmundella bitentaculata*, appears to be eurythermal, living at temperatures ranging from 30° to −1° C. and at depths between 1,500 fathoms and the surface, and even among the widely ranging Siphonophoræ, Bigelow (1911) is able to name only one doubtfully eurythermal species among the 90 known forms.

A study of the temperature reactions of the only known eurythermal scyphomedusa, *Aurellia aurita*, is of interest, for it shows that the success of this species in adjusting itself to a wide range of temperature is due to a remarkable ability for acclimatization. Thus, the *Aurellia* at Halifax, Nova Scotia, ceases to pulsate at 29.4° C., at which temperature it is most active at Tortugas, Florida; and conversely, the Tortugas medusæ cease to pulsate at about 7.75° C., while those from Nova Scotia continue to move at a temperature of −1.4° C. with ice floating in the water above them. If frozen solidly into the ice the Tortugas medusæ are killed, but (as was observed by Romanes, 1885), our northern *Aurellia* withstands this treatment without serious injury.

Aurellia aurita is more abundant in cold than in warm seas, however, perhaps on account of the more abundant zoöplankton and food-supply in cold waters; yet it may be due to a better adjustment to environmental temperature in the medusæ of the cold waters, for at Halifax the surface-temperature in summer is about 14° C. and the range in activity of the medusa is then about 15° C., both above and below this temperature, the animals being most active at from 18° to 23° C. On the other hand, at Tortugas the average surface-temperature in summer is about 29° C., being practically identical with that of the animal's greatest activity; but a rise of 8° C. would cause all movement to cease and is generally fatal to the medusæ, while the same species at Tortugas can still pulsate even at a temperature of 21° below that of the ocean in which they live. It appears probable that *Aurellia* is a boreal or arctic animal which has wandered into the tropics and become fairly well acclimated, although living in these warm waters within 9° C. of its death-temperature. Thus, in comparison with their northern relatives, these Aurellias in the tropics are poorly adjusted to their temperature environment, and a change of even 2° or 3° above their normal temperature causes a decided lassitude and loss of rate in pulsation. We see, therefore, that the medusa has become adapted to a tropical environment, but this has been accomplished at the expense of its factor of safe adjustment to temperature changes.

Harvey (1911, p. 34) showed that *Cassiopea*, which is strictly confined to the tropics, can not withstand a higher degree of heat than can *Limulus* at Woods Hole, Massachusetts. Thus, *Cassiopea* is an animal living constantly within 15° C. of its death-point, yet it is not adapted to withstand higher temperatures than the heart of a northern animal (*Limulus*) living at 25° to 30° C. from its death-point.

In general, one finds that tropical marine animals normally live at temperatures much nearer their death-point than do northern forms. For example, the most resistant reef coral, *Siderastræa radians*, which lives at about 28° to 30° C., is killed at 38.5° C., and the West Indian reef Madrepores are killed at about 35.8°. The tropical sea-urchins, *Diadema* (*Centrechinus*) *setosum* and *Toxopneustes* (*Lytechinus*) *variegatus*, and the brittle-star, *Ophioderma appressa*, are killed at 37.4° to 37.7°. In these forms the temperature of the sea-water in summer is only from 7° to 10° C. below the death-temperature. By way of contrast, we may cite the cases of the arctic forms *Cyanea arctica* and *Beroë cucumis*, which are killed at about 27° and 30° C., respectively, and which live in an ocean not warmer than 14° C., thus being at least 13° to 16° C. from their death-points.

This low factor of safety in tropical marine animals may at times become of biological significance. Thus on July 21–22, 1911, at Tortugas, Florida, after several hot, calm days, the shallow water over Bird Key Reef rose to 33° to 38° C. and Dr. L. R. Cary observed that large numbers of *Diadema, Octopus, Fissurella,* and other mollusks and small fishes were killed in considerable numbers over extensive areas, and corals were injured even when not exposed to the air.

In order that a marine animal may live throughout the year in the shallow or surface waters of the tropics, it must be capable of surviving at 29° C. Similarly, the animals of the Arctic Ocean must survive at 0° and, indeed, *Cyanea arctica* continues to pulsate even when half its bell is frozen into the ice, and after being embedded solidly for several hours it revives at once, apparently uninjured, when the ice has melted.

It is easy to see why such forms as the rhizostomous scyphomedusa *Cassiopea* and the sea-urchin *Diadema setosum* are confined to the tropics, for they lose all power of movement at from 10° to 12° C. Also the floating barnacles, *Lepas fascicularis*, can not survive in arctic waters, for they are unable to move if cooled to 4.6° C., and, conversely, *Cyanea arctica* can not enter the tropics, for it dies at 27° C.

On the other hand, even the tropical *Limulus polyphemus*, from the Marquesas, Florida, survives being frozen into the ice, and near the northern limit of its range, off the northern coast of Massachusetts, it continues to move until heated to at least 40°. Judging from its marked adaptability to extremes of temperatures, one would expect this animal to be of world-wide distribution, yet it ranges only from Maine to Yucatan and is unknown from European coasts.

When a boreal or arctic animal such as *Aurellia* becomes acclimated to the tropics, its upper death-temperature is raised, and conversely it becomes unable to withstand a degree of cold in which its northern relatives may thrive. Its optimum temperature is, however, raised even more conspicuously than its death-points and thus its factor of safety against abnormally high temperatures is reduced.

These facts are illustrated in fig. 8, page 18, wherein the ordinates of the curve *ABC* represent the average rates of pulsation of *Aurellia* at Halifax, Nova Scotia, the abscissas representing the respective temperatures in degrees centigrade. The curve *DEF* shows the same factors for the *Aurellia* from Tortugas, Florida, and it is evident that at Halifax a wide range of temperature, from 2° to 19° C., produces but little change in the rate of the medusa, whereas at Tortug a the medusa lives constantly at the temperature of its maximum activity and any further elevation of temperature causes a marked decline in its rate of pulsation.

The case of the brittle-stars *Ophioderma brevispina* and *O. appressa* is unlike that of *Aurellia*, for at Montego Bay, Jamaica, they both have the same temperature-range, their movements ceasing at 7° to 8° C. in cooled and 37.3° to 38° C. in heated sea-water; yet the former species ranges from Woods Hole, Massachusetts, to the West Indies, and the latter is restricted to the tropics.

An even more striking case is that of the tropical reef-flat coral *Siderastræa radians*, which, although it normally lives in water of 29° to 31° C., can survive without apparent injury after being placed for 8 hours in water at 6.7° C., and one specimen survived with some maceration of tissue a temperature of 1.9° C. It is difficult to see why this coral does not invade the waters of the temperate regions, but it is unknown north of Bermuda.

The development of the ability to withstand higher or lower temperatures is associated with physical changes in the protoplasm. For example, Dallinger (1887), Davenport and Castle (1895), Greely (1901), McGill (1908), Rautmann (1909), etc., observed that organisms when heated excrete water and thus their albumens become denser and the temperature of coagulation is raised. A decided congelation of the slime is observed in *Cassiopea* which has been cooled to its non-active limit.

In the tropical scyphomedusa *Cassiopea xamachana*, however, movements cease long before heat-rigor develops, and in fact the loss of movement is due to a general *muscular* relaxation, which develops several degrees below the temperature at which the nerves cease to transmit the pulsation stimulus. If, for example, we cut a ring (fig. 1) of subumbrella tissue of *Cassiopea*, leaving a radial strip projecting from the outer side and then start a pulsation-wave in the ring, every time the wave passes the point *B* a portion of the wave is diverted and passes out to the end of the strip *BC*. If, then, we place the strip *BC* and the ring in separate dishes of sea-water, and warm the ring while the strip remains at a normal temperature, the amplitude of pulsation is reduced to insensibility in the ring at about 38.5° C., and all the muscles are relaxed while the nervous impulse which produces pulsation still causes the strip *BC* to respond vigorously every time it passes the point *B* up to about 41.7° C. Thus the muscles are more profoundly affected by the heat than are the nerves.

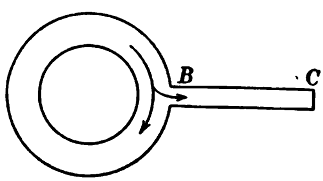

FIG. 1.

There is another reason for the belief that heat-rigor, or at least coagulation of albumens, is not the cause of death at high temperatures, for practically no marine animals can withstand 46° C., most of them dying below 40°, while the most readily coagulated emulsion of egg albumen does not congeal below 56° C.[1]

Snyder (1907, Archiv für Anat. und Physiol., p. 113), basing his conclusions upon Nicolai's observations of 1901, came to the conclusion that as many physiological reactions have the same temperature coefficient as have chemical reactions, the underlying cause of the physiological reactions must be of a chemical rather than a physical nature. In 1908 Snyder published an extensive and important paper upon this subject in which he made use of the results of all previous studies bearing upon the case; and he concludes that the temperature coefficients of all physiological reactions in which metabolism occurs are of the magnitude of the coefficients for chemical reactions, being about 2 or 3.

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[1] As a result of our recent experiments upon the corals of the great Barrier Reef of Queensland it appears that high temperature causes death through asphyxiation, the oxygen of the sea-water becoming insufficient to support the increased metabolism.

The equation for chemical reactions at increasing temperatures may be expressed in the form $y = ac^x$, where y is the rate of the reaction at any 10° C. temperature interval, a is the rate of reaction at the lowest temperature in the series; c is the temperature coefficient, the value of which is usually between 2 and 3; and x is the number of the 10° C. temperature intervals. Thus, if the temperature be raised 10° C., then $x = 1$; if the temperature be raised 30° C. above the lowest temperature in the series, then $x = 3$. Hence, x is the increment of increase in temperature expressed in terms of units of 10° C.

Harvey (1911) tested this subject for the rate of nerve-conduction in *Cassiopea* by experimenting upon two subumbrella rings which were deprived of rhopalia and set into pulsation by means of an entrapped circuit-wave. In being heated from 18° to about 30° C. the rate of pulsation increased in arithmetical proportion to the increment of temperature, in other words, as a rectilinear function of the increase in temperature, not as an exponential function as demanded by Snyder's hypothesis.

As Harvey was able to obtain only one wholly satisfactory series of observations, it seemed desirable to repeat his experiments. This has been done upon five separate subumbrella rings of *Cassiopea*, the results being presented in table 1, which gives the rates of pulsation of five rings of subumbrella tissue of *Cassiopea xamachana* warmed in sea-water, at Tortugas, Florida.

TABLE I.

Temperature of the sea-water.	Number of times per minute the wave traveled around the ring.				
	Ring A.[1]	Ring B.	Ring C.	Ring D.	Ring E.
°C.					
17.5	54.5	......	......	......	......
20.2	60	......	......	......	......
21.6	70	......	......	......	......
24.5	82.5	......	......	......	......
26.6	101.5	......	......	......	......
27	105	......	......	......	......
27.2	......	......	......	127	......
27.8	......	100	......	......	......
28.9	111	......	......	......	......
29	......	......	......	......	140
29.3	......	......	87	......	......
30.9	121.5	......	......	......	......
31.25	......	......	......	163.5	......
32.25	......	......	101.5	......	......
32.82	......	......	......	158	......
32.95	131	......	......	......	......
33.15	......	......	102.5	......	......
34.12	......	......	......	174	......
34.15	......	......	105	......	......
34.3	......	126	......	......	......
°C.					
35.07	......	......	105	......	......
35.1	140	......	......	......	......
35.22	......	......	......	180	......
35.47	......	......	......	......	164
35.95	......	......	......	180	......
36.05	......	......	105.5	......	......
36.3	......	136	......	......	......
36.75	153	......	......	......	......
36.85	......	......	......	183	......
27.03	......	......	103	......	......
37.05	......	......	......	......	154.5
37.4	......	134	......	180 [2]	......
37.65	......	......	124.5	......	......
37.7	......	164	......	......	......
37.9	167	......	......	......	150
38.2	......	155 [3]	......	......	......
38.3	166	......	......	......	......
38.5	......	......	122	......	143 [5]
38.6	......	......	......	......	......
39	173 [4]	......	......	......	......

[1] Ring A was first cooled from 27.3° to 17.5°, its rate becoming lower as follows: 27.3° C., 70 per minute; 26°, 68 per minute; 24.5°, 72 per minute; 22°, 67.5 per minute; 20.3°, 64 per minute; 17.5°, 54.5 per minute. (See Record 1A.)

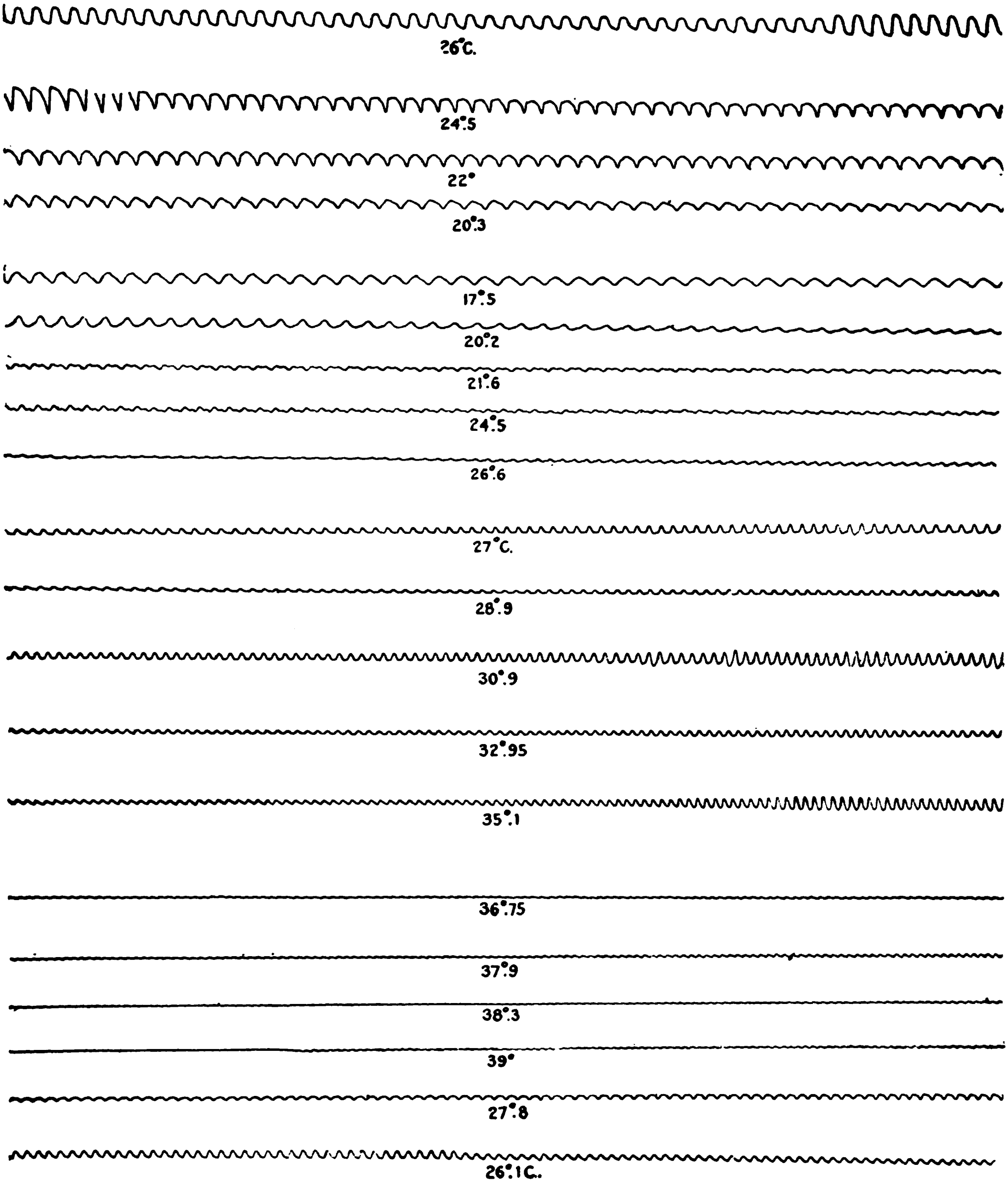
[2] This ring was finally heated to 38.75° and then upon being cooled to 28.5° it gave 89 pulsations per minute, being somewhat weaker than the normal.

[3] Upon being cooled to 30.5° this ring gave 126 turns per minute. Pulsating normally.

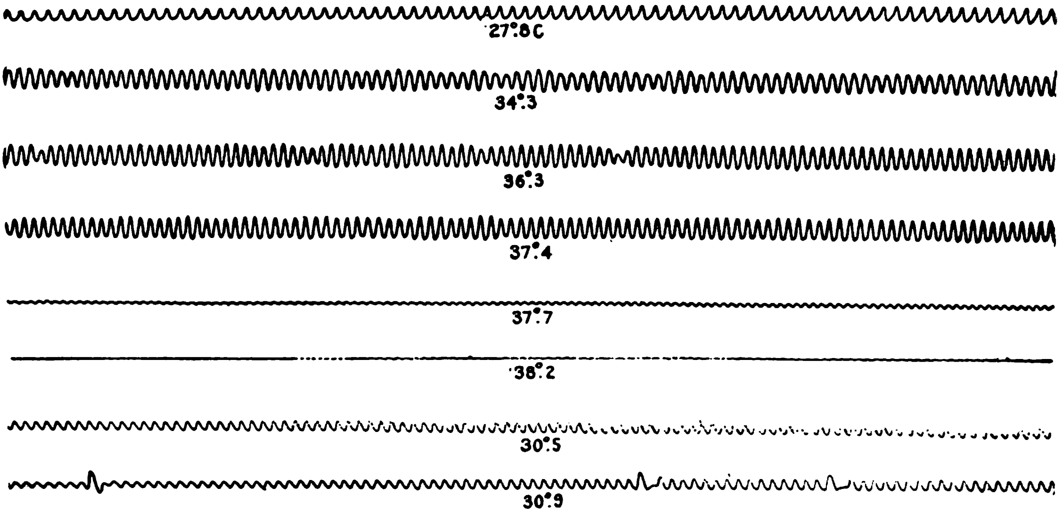
[4] Upon being cooled to 26.1° this ring gave 116 per minute. Fully recovered.

[5] Upon cooling to 29.05° the rate became 120.5°.

The most successful experiment was upon Ring A, the wave within which traveled around the ring 70 times per minute at 27.3° C., and gradually declined to 54.5 at 17.5° C., from which temperature it was slowly heated to 39° C., its rate then becoming 173. Then, upon cooling the ring to 26.1° C., it pulsated in a normal manner at the rate of 116 per minute, showing no ill effects from the experiment (see Record 1A). The obser-



Record No. 1 A.



RECORD No. 1 B.

vations are plotted in figure 2, and it appears that the increase in rate is in the form of a straight line from 17.5° C. to 35° C. In other words, it is arithmetically proportional to the increase in temperature.

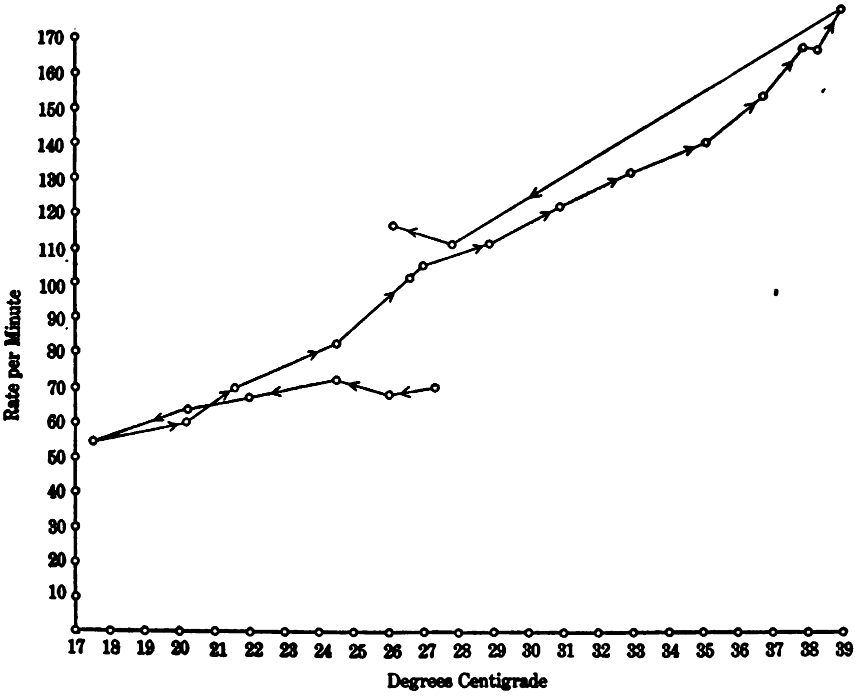


FIG. 2, illustrating Record No. 1 A.

This result is confirmed by taking the average of all five rings; for here also the increase in rate is in the form of a straight line practically coinciding with that displayed by Ring A. This average curve is shown in figure 3. The

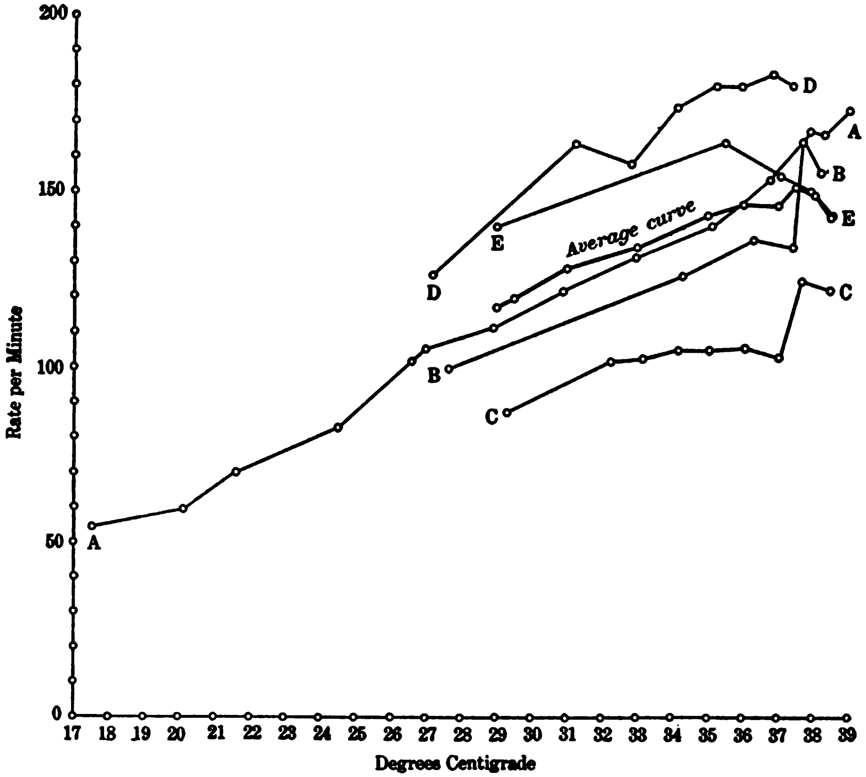


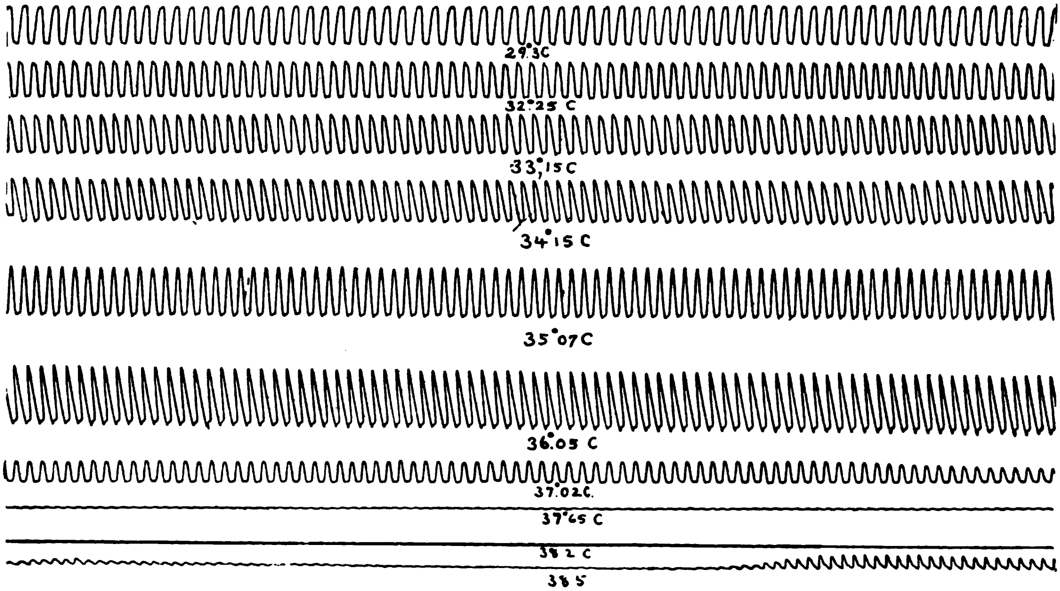
FIG. 3, illustrating Table I.

equation for the increase in rate with elevating temperature between 17.5° to 35° C., in these rings, is $y = 4.85x + 54.5$ and for Harvey's ring it is $y = 6.66x + 50$; where y is the rate at any given temperature above 17.5°, but below 30° in Harvey's and 35° on my curves. x is the temperature-increment. Thus at 25.5° C. $x = (25.5 - 17.5) = 8$, and at 27.5° C. $x = 10$, etc. The constants 54.5 and 50 are the rates of the rings at 17.5° C.

Above 33° C., Harvey observed a sudden decline in rate, but my rings did not display this decline until they had been heated to 35.5° to 39°, and Ring A did not display it even at 39° C.

As Harvey states, these curves bear a close, although possibly only a superficial, resemblance to those of enzyme actions.

Certainly our observations are not in accord with the conditions necessitated were the rate of the nervous impulse a simple chemical reaction, for in this case the curve would be given by an equation of the form $y = ac^x$, where a is the rate of 17.5° C., and c is the temperature coefficient. The actual equation is, however, of the form $y = a + x(c + 1)$.



RECORD No. 1 C.

In this connection it is interesting to observe that Knowlton and Starling (1912) find that in the excised hearts of dogs and cats the increase in the rate of pulsation between 24° and 40° C. is in a straight line, the increase in rate being arithmetically proportional to the increment of temperature. They also comment upon the inadequacy of Snyder's hypothesis to meet the conditions of their determinations, and this is the more remarkable because many physiological reactions do unquestionably follow Snyder's law.

A relation which may possibly be of some significance in this connection is that the kinetic energy of the sea-water increases, as does the absolute temperature, the increment of increase being arithmetically proportional to the temperature increment, thus increasing in a simple linear ratio, as does the rate of pulsation. The kinetic energy increases, however, at a much lower rate than does the rate of nerve-conduction. Thus, between 17.5° and 35° C. the rate of nerve-conduction has increased in an arithmetical ratio from 54.5 to about 140, or to 2.568 times its initial rate.

The kinetic energy has in the same temperature interval increased from K to

$$\frac{273 + 35}{273 + 17.5} K,$$

or from 1 to 1.06. Thus the rate of nerve-conduction has increased from

54.5 to 140, or 85.5 per minute, the kinetic energy in the same interval having increased by 0.06.

The equation for the rate of nerve-conduction at any temperature between 17.5° C. and 35° C. may therefore be expressed by

$$y = \frac{85.5}{0.06}\left[\left(\frac{273 + 17.5 + x}{273 + 17.5}\right) - 1\right] + 54.5$$

or

$$y = 1425\left[\left(\frac{290.5 + x}{290.5}\right) - 1\right] + 54.5$$

or, adopting general terms,

$$y = 1425\left[\left(\frac{T_1 + x}{T_1}\right) - 1\right] + 54.5.$$

Wherein T_1 is the absolute temperature of the lowest point of the series and x is the temperature increment corresponding to the rate y, as has been explained on page 11.

If we let 1 represent the rate of nerve-conduction and also the kinetic energy of the solution at the lowest temperature of the series, then:

$$\frac{\text{The increase in rate}}{\text{The increase in energy}} = \frac{2.568 - 1}{1.06 - 1} = 26.13.$$

Thus the increment of increase in rate of nerve-conduction, for any elevation in temperature between 17.5° and 35° C., is 26.13 times the increment of increase in kinetic energy of the solution.

Hitherto we have been considering the rate at which the nerve-net conducts the stimulus which produces muscular contraction. In nature the stimuli which produce the nerve-impulse arise in the motor centers or marginal sense-organs of the scyphomedusa and, as explained by Mayer, 1908, p. 119, the pulsation-waves annul themselves after each systole; thus the rate of pulsation in the normal medusa is much lower than the rate of nerve-conduction, being, indeed, determined solely by the rate at which the motor centers can generate successive stimuli. In the paralyzed ring, on the other hand, the rate of pulsation is simply that of nerve-conduction, for no new stimuli are generated.

The effect of temperature upon the perfect medusa is therefore its effect upon the stimulus-generating ability of the motor centers—in other words upon the rate of the metabolic process which produces stimulation. We have, then, in *Cassiopea* a unique opportunity for differentiating between the rates of production and that of the conduction of nervous impulses.

Cassiopea xamachana with motor centers intact pulsates at its maximum rate at about 33° C., ceasing to pulsate if cooled to 16.6° or if heated to 38.5°.

Table 2 gives the mean rates of pulsation derived from the study of 7 separate medusæ (with marginal sense-organs intact) at various temperatures, the rates stated in the table being the arithmetical mean of the observed rates. Thus if 3 medusæ each pulsated at the rate of 3 per minute, and if 4 others each pulsated at the rate of 10 per minute, the average rate of pulsation would be 7.

TABLE 2.

Temperature of sea-water.	No. of pulsations per minute.	Temperature of sea-water.	No. of pulsations per minute.	Temperature of sea-water.	No. of pulsations per minute.
° *C.*		° *C.*		° *C.*	
16.6	0	24	18.8	32	31
17	2.5	25	21.96	33	33.9
18	5.5	26	23.4	34	33.25
19	9.6	27	25.3	35	30.25
20	12.7	28	28.2	36	32.4
21	15.8	29	30.8	37	23.06
22	17.4	30	31	38	13.8
23	18.2	31	30.9	38.5	5.25

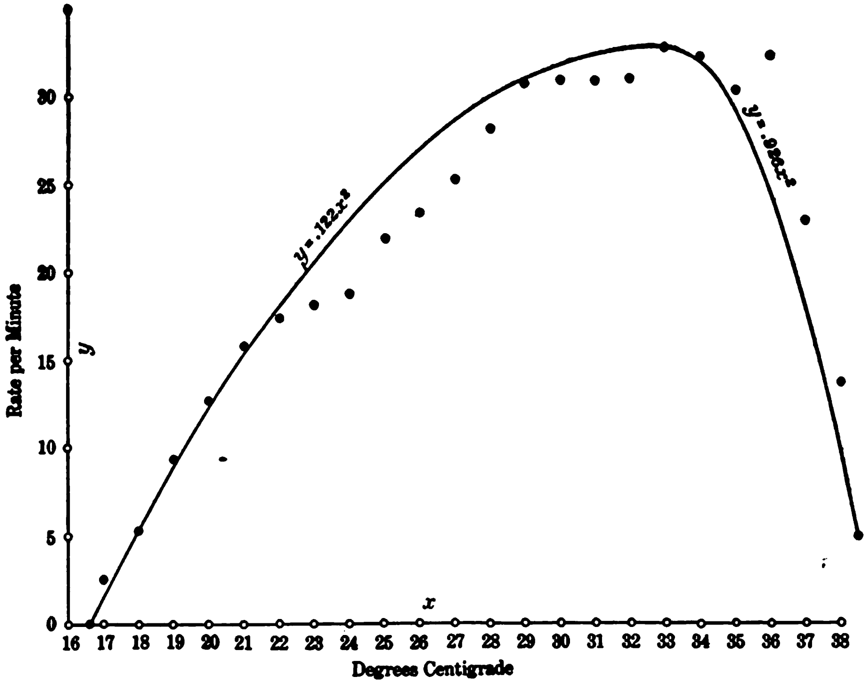
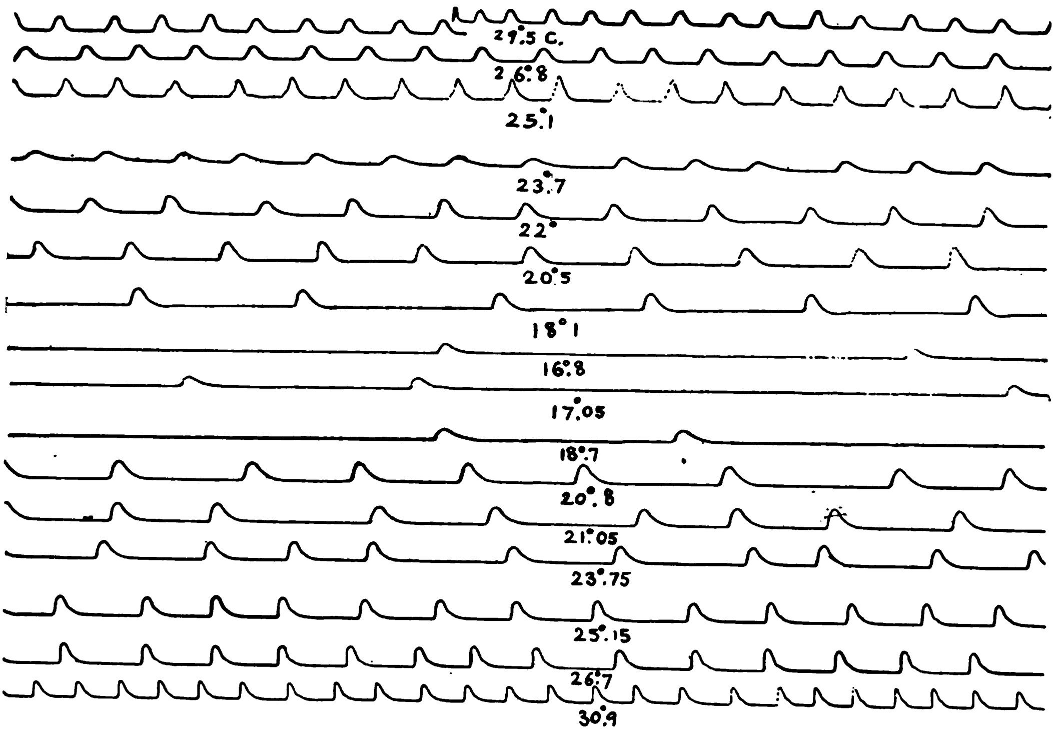


FIG. 4, illustrating Table 2.

The observations recorded in table 2 are plotted in figure 4, wherein it appears that the rate at which the motor-centers engender stimuli declines if the medusa be cooled or heated from 33°. In both heating or cooling the loss of rate is slight at first, but constantly augments as we continue to cool or heat the animal. In fact, the loss of rate is about proportional to



RECORD NO. 2.—A *Cassiopea xamachana* cooled from 29.5° to 16.8° C. and then warmed to 30.9° C.

the square of the departure of the temperature from its optimum of 33°. Thus a change in temperature above or below the optimum appears to reduce the rate of pulsation in proportion to the square of the change. For example, if cooling 1° C. produces a loss of rate of 1 per minute, a cooling of 2° from the optimum will produce a loss of 4, and for 3° the loss will be about 9, etc.

Thus if x be the departure of temperature from the optimum of 33° (for example at 30° or at 36°, $x = 3$), and if y be the loss in rate (for example, if the rate has declined from 34 to 30, $y = 4$), then in cooling $y = 0.122x^2$, and in heating $y = 0.926x^2$.

It seems that the loss of rate in heating is nearly 8 times as rapid as in cooling. Moreover, when the medusa has lost all movement through being heated, its muscles are relaxed and recovery rarely occurs, even if the medusa be then cooled to a normal temperature. If, however, the medusa be cooled to a standstill, the muscles still exhibit a decided tonus, some shrinkage occurs, and the slime congeals into a gelatinous mass.

The falling-off in rate that is observed when the medusa is either cooled or heated from its optimum temperature appears not to be a simple chemical reaction, for it is more rapid than can be represented by the equation

$y = ac^x$, but in all these experiments the equation $y = bx^2$ fits the observed facts fairly well, but this formula is merely tentative and should not be taken as being more than an empirical attempt to express the law. If this be the law, however, it would mean that the decline in rate varies as the square of the degree of heating or cooling above or below the optimum, and it leads one to suspect either that an inhibiting enzyme is formed in a constantly accelerated ratio or a stimulating one is destroyed in the same manner. This is, however, a mere suggestion, for the observations are too few to establish it as a law.

Experiments upon *Cassiopea frondosa* and *Aurellia aurita* at Tortugas appear to lend some support to the results attained from a study of *C. xamachana.*

Thus table 3 shows the average rates of four medusæ of *Cassiopea frondosa* at Tortugas, Florida, cooled from 28.5° to 12.5° C., and figure 5 gives a graphic representation of the results. It is evident that the rate declines somewhat more slowly than if it followed the equation $y = 0.0507x^2$.

TABLE 3.

Tempera-ture.	Pulsations per minute.
° C.	
28.5	13
25	11
20	9
15	3
12.5	0

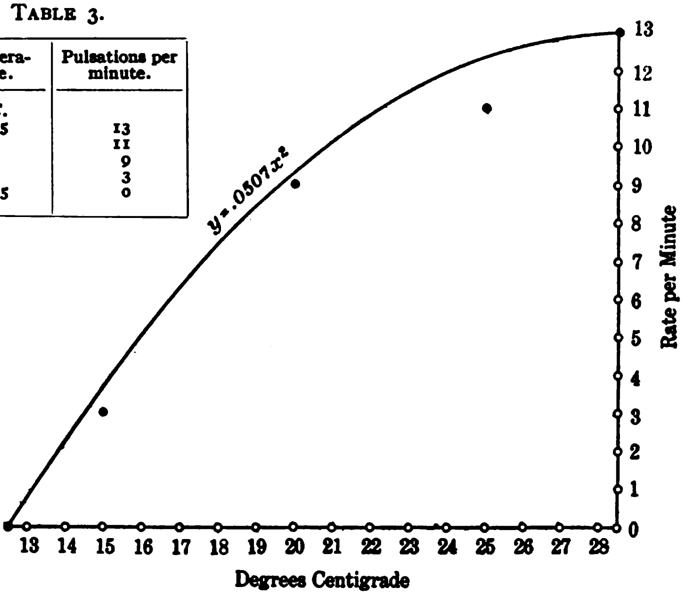


FIG. 5, illustrating Table 3.

Upon being heated, *Cassiopea frondosa* begins to lose in rate at 32.45° and all movement ceases at 38.3° C. Unfortunately, I did not make enough observations to determine the curve of the decline, but if it be a parabola its equation must be $y = 1.05x^2$, and thus the decline in rate upon heating above the optimum is about 20 times as rapid as the decline on cooling below the optimum.

Table 4 and figure 6 show the decline in rate of an *Aurellia aurita* from Tortugas, Florida, cooled from 28.8° to 11.8° C.

TABLE 4.

Temperature of sea-water.	Pulsations per minute.	Temperature of sea-water.	Pulsations per minute.
° *C.*		° *C.*	
28.8	25	14.6	12
20.5	18	13.2	8.5
18.1	17	13	1
16.4	14	11.8	0

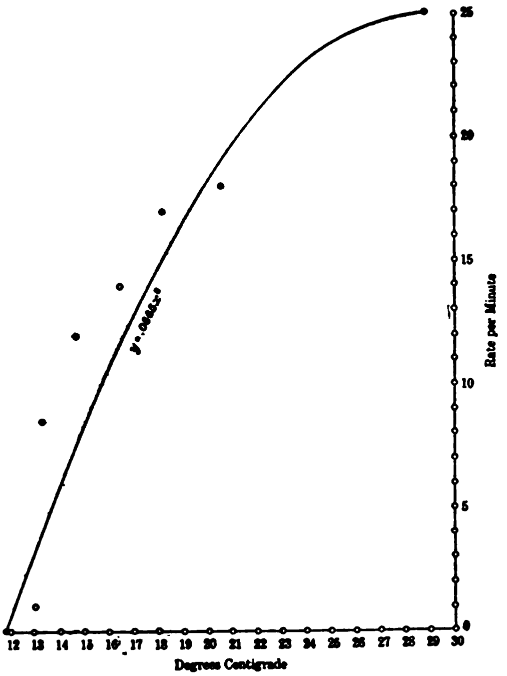


FIG. 6, illustrating Table 4.

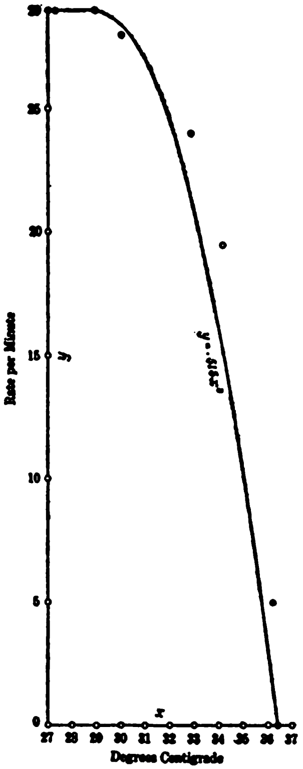


FIG. 7, illustrating Table 5.

TABLE 5.—*An Aurellia aurita at Tortugas, Florida, warmed from 27.3° to 36.4° C.*

Temperature of sea-water.	Pulsations per minute.	Temperature of sea-water.	Pulsations per minute.
° *C.*		° *C.*	
27.3	29	34.1	19
28.9	29	36.15	5
30	28	36.4	0
32.8	24		

The parabola $y = 0.0865x^2$ fits the observed facts fairly well.

Similarly, table 5 and figure 7 show the decline in rate displayed by a specimen of *Aurellia aurita*, from Tortugas, which was heated from 27.3° to 36.4° C., and the actual conditions are fairly well represented by the curve $y = 0.515x^2$, showing that in heating the rate declines about 6 times as rapidly as it does on cooling; the case being in general similar to that of *Cassiopea xamachana*, where the rate of decline upon heating is about 8 times as rapid as in cooling.

Hitherto we have considered only tropical forms or animals living in the tropics, and in these we see that a definite temperature of maximum activity is well shown in their temperature reactions. Thus for *C. xamachana* the optimum is about 33° C., for *C. frondosa* 28.5° to 32.5°, for *Aurellia aurita* 29°, for the movements of the branchial arms of *Lepas fascicularis* it is about 32° C., and for the reef corals it ranges between 30° and 35.7° C. Thus the optimum temperature is very close to the usual temperature of the sea-water itself, this being about 28° to 31° in summer. Even a slight degree of cooling or heating beyond the optimum causes a decided falling-off in rate.

A very different picture is presented by the arctic scyphomedusa *Cyanea arctica*, from Halifax, Nova Scotia, in September, for here the optimum temperature ranges from about 2° to 21° C., the animal's rate of pulsation increasing only slightly as the temperature rises, and with an ill-defined maximum at about 19° C. Upon cooling below 2° C., the medusa pulsates until the ice imprisons it, although its rate declines rapidly. Similarly, if we heat it above 21° C. the rate declines at about the same rate as it does upon cooling from 2° C. All movements cease at about 27° C.

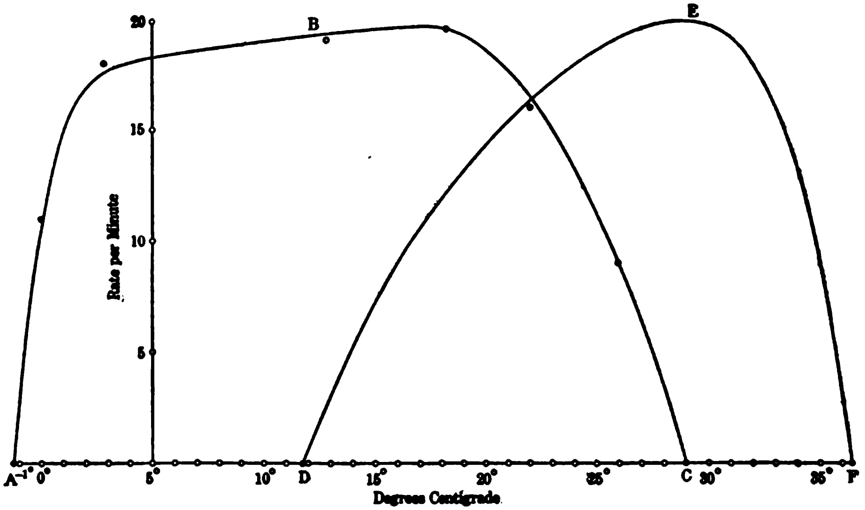


FIG. 8.

The same general conditions are also shown by *Aurellia aurita* from the cold waters of Nova Scotia, in marked contrast with its behavior at Tortugas. For example, at Halifax, Nova Scotia, in September, when the harbor water is about 14° C., the optimum temperature for *Aurellia* is anywhere from 2° to 18° C. and it ceases to pulsate at −1.4° and at 29.4° C.

At Tortugas, on the other hand, a very slight departure in temperature either above or below 29° C. causes a decided falling-off in rate.

The contrast between the behavior of *Aurellia* at Tortugas and at Halifax is shown in figure 8 wherein the ordinates represent average rates of pulsation and the abscissæ temperatures, the curve *ABC* applying to the medusæ from Halifax, while *FDE* shows the general reactions of the same species from the warm waters of Tortugas, Florida.

The wide range in temperature in which these northern forms are about normal or only slightly increasing in activity accords with the fact that tropical marine animals are, physiologically speaking, but poorly adjusted to their temperature environment in comparison with creatures of colder waters, for the tropical forms live near their upper death-points and slight elevation in temperature affects them adversely. Nor are they capable of withstanding the same proportionate degree of cold as do northern forms, as may be seen after every severe "norther" in winter, when the Florida beaches are strewn with reef fishes which have succumbed to the cold, although the temperature of the water over the reefs in these storms rarely declines below 17° C.

TABLE 6.

Name of coral.	Temperature at which death occurs.	Lowest temperature survived without apparent injury.	Temperature at which tentacles were most fully expanded.	Temperature at which tentacles retracted and movements ceased.	Highest temperature survived without apparent injury.	Temperature at which death occurs.
	°C.			°C.	°C.	°C.
Siderastræa radians[1].......	4.5	1.9 to 6.7	32.5 to 35.7	35.7	38.2	38.5
Siderastræa siderea[2].......	5.1	11.3	....	....	37.8	38.3
Mæandra æreolata[3].......	....	10.2	30 to 34.2	31.8 to 35.2	35.9	36.7
Mæandra clivosa[4].......	....	15.3	31.3	34.4	37.7	38.2
Manicinia gyrosa.......	....	....	....	....	36.1	37.8
Porites furcata[5].......	....	3 hrs. at	32.65	36.4	38.2	37.2 to 38.3
Porites clavaria[6].......	....	10.2	32.8	34.5 to 34.9	36.7	37.7
Porites astræoides[7].......	15.3	....	33.7	34.3	36.7	37.7
Favia fragum[8].......	....	....	....	34.7	36.1	37.2
Orbicella annularis[9].......	14.1	16	....	....	36.2	36.8
Orbicella cavernosa.......	above 8.4	....	....	....	....	below 37.3
Agaricia sp.......	....	....	....	....	....	35.95
Mussa (Isophyllia) dipsacea.	....	....	....	....	....	36.7
Mussa (Isophyllia) rigida...	....	....	....	....	....	35.95
Oculina diffusa.......	....	15.6	....	....	36.1	36.4
Madrepora (Acropora) muricata[10].......	....	14.1	33.75	34.7	36.8	35.8 to 37
Madrepora (Acropora) palmata.......	....	....	33.75	34.7	35.7	35.8
Eusmilia aspera (knorri)...	17	....	....	....	36.1	36.4

[1] Survives without apparent injury after being for 9 hours at 11.2° to 12.1° C.
[2] Survives 13° to 14.4° C. for 9 hours; half killed by 11.2° to 12.1° for 9 hours.
[3] Apparently uninjured by 13.2–15.1° for 10.5 hours.
[4] Killed by 13° to 14.4° for 9 hours.
[5] Nearly killed by 13.2° to 15.1° for 10.5 hours.
[6] Not injured by 13.85° to 16.8° for 9 hours.
[7] Killed by 14° to 15° for 9 hours.
[8] Injured but survived 13.85° to 16.8° for 9 hours.
[9] Killed by 14° to 15.8° for 9 hours.
[10] Killed by 13.3° to 15° C. for 9 hours.

The reef corals are interesting, for, as is well known, they are restricted to tropical seas. Accordingly, the vital limits in respect to temperature of the 18 most abundant reef species of the Florida-Bahama region were studied.[1] The results are presented in table 6, the method pursued being the same as in all other experiments recorded in this paper: the corals were placed in large glass aquaria and the temperature was raised slowly at the rate of about 2° C. per hour, with frequent stirring of the water. Time is an important factor in these experiments, for animals can withstand a higher degree of heat if the temperature be raised quickly than if it be raised slowly. These experiments were made during May–July 1912, at Golding Cay, Andros Island, Bahamas, and at Tortugas, Florida, when the normal temperature of the sea-water ranged from 27° to 29.5° C. as shown in table 6.

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[1] Experiments upon corals of the Great Barrier Reef of Australia, near Torres Straits, show that their temperature reactions are on the whole similar to those of the corresponding Atlantic genera. Thus natural selection has not aided the Florida corals in withstanding cold, or improved the resisting powers of the Pacific forms in respect to heat.

It appears that the reef corals at Tortugas, Florida, live in water which is commonly within 10° C. of their upper death-temperature, and if the ocean were heated to 38° C. (100.4° F.) only one species, *Siderastræa radians*, could survive. This form lives on the shallow flats, often in places where the circulation is imperfect and where wide temperature ranges occur. Accordingly, it is also the species most resistant to cold, withstanding 6° to 7° C. without apparent injury, but usually being killed at about 4.5° C. (40° F.), although one individual survived without apparent injury after being at a temperature of 1.9° C. (35.4° F.) for 11 hours.

Next to *Siderastræa radians* the most resistant coral is *S. siderea*, although it lives in relatively deep water on the outer reefs, where the circulation is of the best and the temperature range is therefore slight. It is associated in its habitat with *Orbicella annularis*, one of the most sensitive of the reef corals, which is killed at 14.1° and 36.8° C.

In general, however, the corals of the shallow-reef flats, such as *Siderastræa radians*, *Porites furcata*, and *Mæandra areolata*, are the most resistant both to heat and cold, while those of deep water, such as *Madrepora palmata*, *Eusmilia knorri*, and *Oculina diffusa*, are the least resistant.

Forms such as *Porites clavaria*, *P. astræoides*, *Mæandra muricata*, *Orbicella annularis*, *O. cavernosa*, and *Favia fragum*, which usually live in fairly shallow but freely circulating water, all show moderate powers of resistance.

The air-temperature at Tortugas commonly ranges from a maximum of 98° F. in summer to about 60° F. in winter,[1] and the coldest "northers" in winter appear to reduce the temperature of the water over the reefs to about 17.2° C. (63° F.). In view of this fact, the more abundant reef species were maintained at about 13.9° C. (57° F.) for periods of at least 9 hours.

As a result, we are led to conclude that were the water cooled by an exceptionally prolonged norther to 13.9° C. for 9 hours, *Siderastræa radians*, *S. siderea*, and *Mæandra areolata* would survive without apparent injury, while *Porites furcata*, *P. clavaria*, *Mæandra clivosa*, and *Favia fragum* would also survive, but with more or less injury, the first-named being the most resistant. On the other hand, this temperature would be fatal to *Orbicella annularis*, *Porites astræoides*, and *Madrepora muricata* (*cervicornis*).

The observations cited above are upon the death-temperatures, but the temperatures at which the feeding reactions and normal metabolic processes cease are much more significant, for naturally an animal can not long survive in water in which it can neither move nor function. Unfortunately, however, no observations were made upon this interesting point; but, crude as they are, the experiments show why it is that most of these reef corals can not enter the waters of the temperate regions and demonstrate that, in common with other marine animals, they live at temperatures within about 5° of their temperature of maximum activity and within 10° of their death temperatures. Thus the factor of safety in respect to elevation of temperature is far less in tropical than in temperate marine animals, and they are, relatively speaking, poorly adjusted in a physiological sense to their temperature environment; slight differences in temperature producing a more serious effect than is observed to result from similar temperature changes with the marine animals of the temperate regions. Moreover, paradoxical as it may seem, tropical marine animals can withstand cooling better than they can survive heating above their normal life-temperature. High temperature appears to cause death by asphyxiation, the oxygen of the sea-water becoming insufficient to support the augmented metabolic activity of the animal.

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[1] In the year from June 1, 1912, to May 31, 1913, the air temperature at Tortugas ranged from 95° to 65.5° F. as determined by a self-recording thermometer.

TABLE 7.—*Reactions to Temperature.*

Name of animal.	Cold.		Temperature of greatest activity.	Heat.		Normal temperature of sea-water in which the animals were living.
	Death occurs.	Movements cease.		Movements cease.	Death occurs.	° *C.*
Aurellia aurita: From Tortugas, Florida, June–July 1911.	Killed if ice crystals form within gelatinous substance of medusa......	7.75° to 11.8° C.......	About 29° C........	36.4° to 38.4° C.....	38.5° C.	29
From Halifax, Nova Scotia, September 1911.	Survives being frozen solidly into the ice.	− 1.4° C. with ice crystals forming in water.............	18° to 23° C........	29° to 29.7° C.......	29° to 30° C.: usually survive if heated not above 29° C.	14
Cyanea arctica from Halifax, Nova Scotia, September 1911.	Survives freezing solidly into ice without apparent injury, being more resistant than *Aurellia.*	Below − 1.4° C. while ice crystals are forming in water.	About 19° C........	26.8° to 28° C.......	26.8° to 28° C........	14
Pennaria tiarella: From Tortugas, Florida, July 1911.	Killed by freezing to −2.3° C.	....	....	....	....	29
From Montego Bay, Jamaica, March.	−0.6° C.	....	....	34.7° C...........	....	22 to 26
From Woods Hole, Massachusetts, Aug. 1911.	Survives freezing to −2.3°.	....	....	....	....	22
Lepas fascicularis from Tortugas, Florida, June–July 1911.	Killed between −1.4° and −2.3° C. while ice crystals form in the water. About half of specimens recover from −1.4° C.	4.6° to 11.5° C........	30.3° to 32.1° C.....	38.4° to 40.7° C.....	42.3° C.............	29
Limulus polyphemus from Marquesas Keys, Florida, July 1911.	Survives −2.3° C. with ice forming in water.	+0.8° C.............	41° C.............	45.7° C............	46.25° C............	30
From Annisquam, Mass., Oct. 1, 1911.	Survives being frozen into a solid block of ice and then thawed out.	Below −1.1° C. slow movements continue while ice crystals are forming in water....	....	40° C.............	41° C..............	16
From Woods Hole, Mass., Aug. 1911.	....	....	38.1° C...........	41° C.............	41° C..............	22
Beroë cucumis, from Halifax, Nova Scotia, Sept. 1911.	....	....	18° to 28° C.......	27.8° to 29.8° C.....	29.7° to 30° C........	14
Cassiopea frondosa, from Tortugas, Florida, July 1911.	8.3° to 9.7° C..........	12.1° to 12.9° C......	About 32.5° C......	38.3° to 40° C.......	40° C...............	29

TABLE 7.—*Reactions to Temperature.—Continued.*

Name of animal.	Cold.		Temperature of greatest activity.	Heat.		Normal temperature of sea-water in which the animals were living.
	Death occurs.	Movements cease.		Movements cease.	Death occurs.	
Diadema setosum, from Tortugas, Florida, July–Aug. 1911.	+2.6° C. The spines recover temporarily when animal is warmed from this temperature, but tube-feet do not recover, so the animal dies.	Tube-feet cease to move at 10° to 10.8° C.; spines continue to move down to 5° C.	Spines are most active at 36.5° C.	Spines cease to move at 36.5° to 37.6° C.; tube-feet cease at 35.5° C.	37.4° to 37.6° C.; spines recover temporarily but tube-feet do not, so animal dies.	°C. 29
Larvæ of Atlantic palolo worm (*Eunice fucata*) 4 or 5 days old, from Tortugas, Florida, July 20 and 21, 1911.	Recovers from +1° to 6° C., but is killed at −2.3° C.	7° to 9.5° C.	About 35.5° C.	41° to 42.7° C.	Above 42.7° C.	29
Toxopneustes (*Lytechinus*) *variegatus*, from Montego Bay, Jamaica, West Indies.	Below 0° C.	7° C.	24° to 36° C.	38° C.	Can not survive 37.7° C.	24.6 to 25.1
Ophioderma brevispina from Montego Bay Jamaica.[1]	− 0.6° C.	7.5° to 8° C.	About 30° C.	37.3° to 37.7° C.	37.7° C.	22.35 to 25.3
Ophioderma appressa.[2] Montego Bay, Jamaica.	....	....	....	37.2° to 37.7° C.	37.7° C.	22.3 to 25.3
Ophioderma brevicauda[2] from Montego Bay, Jamaica.	Survived 1.2° C.; killed at −1.7° C.	7.5° to 8° C.	....	....	....	....

[1] This species ranges from Woods Hole, Massachusetts, to West Indies. [2] This species is confined to the tropics.

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II.

THE RELATION BETWEEN THE DEGREE OF CONCENTRATION OF ELECTROLYTES OF SEA-WATER AND THE RATE OF NERVE-CONDUCTION IN CASSIOPEA.

BY ALFRED GOLDSBOROUGH MAYER,
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Thirteen figures.

THE RELATION BETWEEN THE DEGREE OF CONCENTRATION OF THE ELECTROLYTES OF SEA-WATER AND THE RATE OF NERVE-CONDUCTION IN CASSIOPEA.

By Alfred Goldsborough Mayer.

CONCLUSIONS.

If sea-water be diluted with distilled water, or with a 0.9 molecular solution of dextrose, thus preserving its normal osmotic pressure but reducing the concentration of the cations of sodium, magnesium, calcium, and potassium, the rate of nerve-conduction increases as dilution proceeds, becoming most rapid in 90 per cent sea-water + 10 per cent distilled water or dextrose. In 80 per cent sea-water it is again about normal in rate, and in successively lowered concentrations it declines in a right-line ratio. The curve for rate of nerve-conduction in sea-water diluted with distilled water is practically identical with that for sea-water diluted with 0.9 molecular dextrose, thus showing that the changes in rate are due to changes in concentration of the electrolytes and not to osmotic pressure. (See fig. 6, page 40.)

If sea-water be diluted with a solution of 0.487 molecular sodium chloride dissolved in 0.075 molecular dextrose, thus preserving the normal concentration of sodium in sea-water and the normal osmotic pressure of the sea-water, the rate of nerve-conduction is most rapid in about 90 per cent sea-water + 10 per cent of this solution and then declines becoming about normal in rate in 80 per cent sea-water + 20 per cent of the (NaCl + dextrose) solution, after which it declines in a right-line ratio upon further dilution, but more slowly than if the sea-water had been diluted with distilled water or dextrose. Thus the sodium cation is an active stimulant for nerve-conduction. (See fig. 7, page 43.)

Similar experiments with the magnesium cation show that it is not a stimulant for nerve-conduction, being no more effective in this respect than is distilled water. Thus it is inert and nontoxic, and exhibits only negative properties and probably it should not be classed as an active inhibitor. Speaking crudely, its rôle in respect to sodium in sea-water is comparable to that of the nitrogen of the air in relation to oxygen. (See fig. 9, page 47.)

In very slight excess the potassium cation produces a permanently stimulating effect, as does sodium, but in denser concentration it produces momentary stimulation of the rate of nerve-conduction followed by depression. In all essential respects the effects of potassium are similar in kind, but more marked in degree, to those of its close chemical ally, sodium.

I can not determine the individual effects of the calcium cation owing to its habit of associating itself with the sodium, thus enabling the sodium to offset the effects of magnesium.

The laws stated above for the rate of nerve-conduction apply also with modifications of detail to the rate at which the motor centers (the rhopalia) generate the stimuli which produce the nerve-impulse. The rhopalia are, however, more readily affected by osmotic and by concentration changes than are the nerves, and I have not observed any increase in rate upon diluting the sea-water with distilled water or dextrose. Any change in concentration either above or below that of normal sea-water produces depression in the action of the rhopalia.

Attention may be directed to an explanation of the apparent converse relation between the activities of muscles and cilia in trochophores, ctenophores, and other forms having well-differentiated cilia which move in a coördinated manner. In all these cases the normal muscular tonus of the animal produces a state of tension over the outer skin, thus pressing upon the cilia-bearing cells and reducing or even stopping their movement. When this tonus is relieved, however, the cilia beat rapidly. Thus magnesium reduces the muscular tonus, but its depressant effect upon cilia is not so marked as upon the muscles, and the cilia-bearing cells being relieved of pressure beat with abnormally great activity in such a solution.

Sodium, on the other hand, contracts the muscles, thus increasing the pressure upon the cilia-bearing cells and stopping the movement of their cilia. Hence this converse relation between neuro-muscular and ciliary movement is a mechanical, not a chemical, matter.[1]

THE GENERAL NATURE OF THE PULSATION-WAVE.

Cassiopea xamachana is a very favorable animal for physiological experimentation, for it normally lives in semistagnant lagoons and is, therefore, relatively insensitive to changes in osmotic pressure due to concentration or dilution of the sea-water. Moreover, as there is probably a considerable amount of dissolved CO_2 in its normal environment, and as the medusa is infested with symbiotic plant-cells, it thrives remarkably well in aquaria, there being practically no deaths due to confinement under laboratory conditions.

In this series of experiments, whenever the animals were placed in a new solution they were permitted to remain in a large quantity of it for at least an hour with several changes before the effect of the new solution was determined by a kymograph record. In this manner the ultimate condition of penetration and balance between the animal and the surrounding solution was secured. The relative concentrations of the solutions were determined by titration with $AgNO_3$ and potassium chromate.

The effects described are all practically reversible, and fatal or seriously injurious degrees of dilution of ions were avoided, so that when the animals were returned to sea-water they soon regained most of their normal rate and amplitude of pulsation.

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[1] Mayer (1912), Ctenophores of the Atlantic Coast of North America, p. 25. Carn. Inst. Wash. Pub. No. 162.

The stimuli which cause pulsation normally arise in the motor centers called rhopalia, or marginal sense-organs, of which there are usually about 16 in *Cassiopea xamachana*, although they may vary in number from 9 to 23. It appears that this normal stimulation is due to the constant formation of sodium oxalate in the rhopalia, thus precipitating calcium oxalate to form the crystals of the sense-club and setting free ionic sodium, which is a most powerful stimulant.[1]

The pulsation-rate of the normal medusa is therefore the rate at which its motor centers can produce successive stages of stimulation. This is always much slower than the rate at which the nerves can conduct the pulsation-wave around the subumbrella of the medusa, and there is a long pause after each contraction, during which the animal remains unstimulated and hence inactive.

Cassiopea is, however, an excellent animal for physiological studies upon nerve-conduction, on account of the ease with which we may dispense with the motor centers and yet maintain a continuous neurogenic pulsation-wave in the subumbrella tissue. This method was devised and described by Mayer, 1906, p. 22, and it consists in cutting off the rhopalia, thus paralyzing the nervous network of the subumbrella. Then the subumbrella tissue is cut into the form of a ring-shaped strip, and a pulsation-wave is started in *one direction* in this ring, so that it must continue to travel around the ring, being in effect entrapped within the circuit of tissue through which it must continually progress in one direction, a single stimulus often maintaining itself in this manner for days at a time.

Such entrapped circuit-waves are of course not peculiar to *Cassiopea*, for they may be started and maintained in ring-shaped strips of the ventricle of the heart of the sea turtle, *Caretta caretta*, in the hearts of fishes, or in other scyphomedusæ. We may thus study the operations of the nervous network as a continuous transmitter of a single pulsation-stimulus.

As has been said, the nerves transmit this stimulus around the bell much more rapidly than the motor centers can engender it. For example, a medusa, which normally gave 45 pulsations per minute, was deprived of its rhopalia and cut into the form of a ring, and a pulsation-wave then traveled constantly around this ring at the rate of 129 turns per minute. This ring also responded without missing a pulsation to 152 Faradaic shocks per minute, and it followed 190 shocks, responding by a separate contraction to each one for about 3 minutes, after which it occasionally failed to respond, following alternate stimuli. Many experiments of this sort were made, always with the same general result, and it is evident that the nerves and muscles can react to successive stimuli with greater frequency and at shorter time-intervals than they are called upon to respond in nature.

It is evident, then, that there is a considerable "factor of safety" in the normal medusa, and that the motor centers initiate pulsation-stimuli at a rate much slower than that to which the nervous network and the underlying muscles can respond, and thus the danger of muscular fatigue is avoided.

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[1] Mayer, 1908, p. 130.

As has been pointed out by Romanes (1885), Bethe (1903), and Harvey (1911), there must be some mechanism of adjustment by virtue of which the wave travels at a faster rate around the rim than at points nearer the center of the subumbrella, the rate being directly proportional to the length of the radius of the zone of pulsation.

In order to determine the average rate of transmission of the pulsation-impulse by the nervous network, eight separate rings were cut, one from each of eight *Cassiopea xamachana*. These rings were each 90 mm. in outside diameter, 67 mm. in inside diameter, and 11.5 mm. wide. Thus the mean diameter of each ring was 78.5 mm. and its mean circumference 247 mm.

TABLE I.

Ring No. on record No. 1.	Diameter of medusa from which the ring was cut.	Times per minute wave went around ring.	Distance wave traveled per second.	Temperature.
	mm.		*mm.*	°
1	98	117	481	30.45 C.
2	110	116	478	28
7	109	113	464	29.7
*6	111	106	436	29.8
3	110	104.5	429.5	29.8
5	97	103	422	29.5
8	108	103	422	30.3
4	115	93.5	384	28.5
Av. condition	107	107	440	28.9

* This ring exhibits *pulsus alternans*. For an explanation of this phenomenon, see G. A. Mines, 1912, Proc. Cambridge Philosophical Society, vol. 16, p. 617.

The results obtained from the kymograph record, figure 1, are stated in table 1, which shows the rate of nerve transmission, in millimeters per second, determined in natural sea-water, ranging in temperature from 28° to 30.45° C.

These 8 medusæ ranged from 97 to 115 mm. in diameter, and their pulsation-waves traveled at rates varying from 384 to 481 mm. per second, the average being 440 at an average temperature of 28.9° C. In these experiments, the rate of transmission bears no relation to the size of the medusa.

All experiments show that, as in other invertebrates, strong stimuli travel faster than weak ones. Thus should a ring stop, we can not start it again and continue the same series of experiments with it, for the new stimulus is almost certain to differ in strength from the former one; or, if all care be taken to make the successive electrical stimuli as nearly as possible identical in strength, they will be received differently by the tissue and the resulting pulsation-waves will differ in rate. Thus a ring must remain in pulsation throughout the entire series of experiments that one performs upon it or no conclusions can be drawn respecting the relative rates of its pulsation-stimulus in the various solutions.

On the other hand, temperature, purity of water, etc., being unchanged, the rate remains nearly constant for any single stimulus. Thus Harvey (1911, p. 131) maintained such a ring in pulsation for 11 days at an average velocity of 774 mm. per second. I once had a ring which remained in pulsation for 6 days, the wave traveling from 200 to 206 times per minute around the ring. The rings live for two or more weeks and exhibit abortive efforts at regeneration, but being without means of obtaining food they gradually decline in size and die.

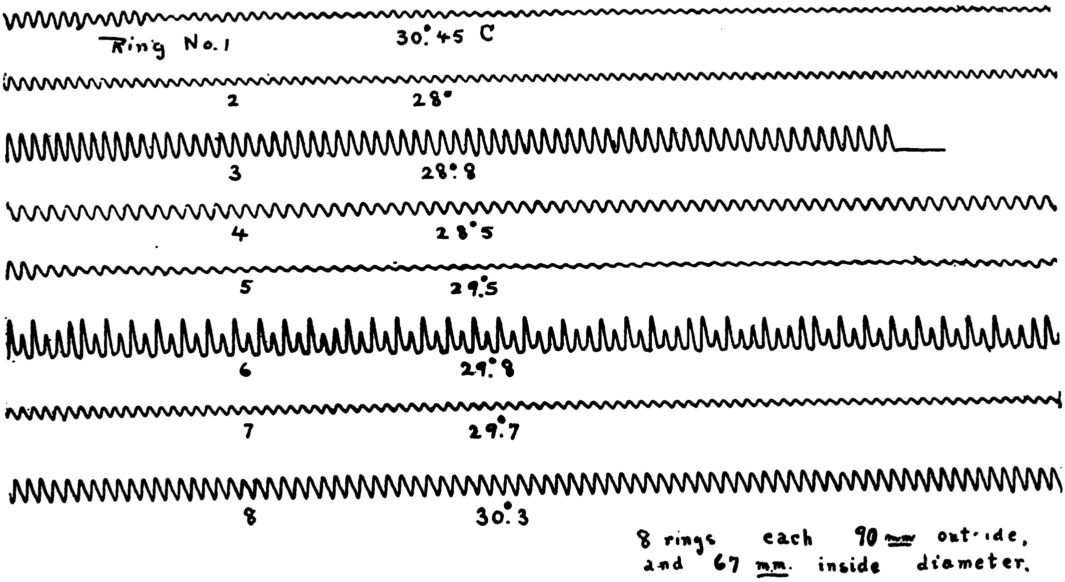

FIG. 1.

These pulsating rings are usually almost machine-like in their uniformity of rate under unchanged environmental conditions; but while the rate of nerve-conduction remains quite constant, the muscles become fatigued and thus the amplitude of pulsation steadily declines, becoming much reduced at the end of a few days. Thus the muscles fatigue more readily than do the nerves.

Kymographic records enable us to study not only the rate, but the form of the pulsation-wave, and this is important; for in order that comparisons may be made it is necessary that the character of the wave should not change throughout the series of tests to which the ring may be subjected in the course of any one set of experiments.

For example, the series of lines on the record (fig. 2), which were obtained from a single medusa, can not safely be compared one with another and the record is therefore worthless, on account of the tendency of the pulsation-stimulus to break up into two waves, the one following the other at the same or at different rates as in lines 1 and 3 from the top of the figure; or, the wave may break up into a complex series of strong and weak wavelets due to spontaneous excitement, as in line 5.

When a simple contraction-wave breaks up into two or more wavelets, the one following the other, the rate declines, for strong stimuli travel faster than weak ones; and if an originally unitary wave divides into two, the stimulus represented by each component wavelet is naturally less than that of the wave as a whole. Thus a ring which gave 168 pulsations per minute with a unitary wave gave only 132 per minute when this wave broke into two component wavelets, the one following the other around the ring. Moreover, if two waves follow one behind the other, the tissue does not enjoy so long a resting-period as if one simple wave were in the circuit, and a certain time must be permitted to pass before the muscles which have responded by a contraction can again fully respond to another stimulus. In some rings the tissue may give a strong response only to every alternate or even every third return of the wave, showing that the muscles which have very recently contracted can not again give a maximal response until a certain resting-time has elapsed. This was first observed in the muscles of Scyphomedusæ by Romanes, 1885, and has been studied in detail by Bethe (1903 and 1908). (See line 6 of fig. 1.)

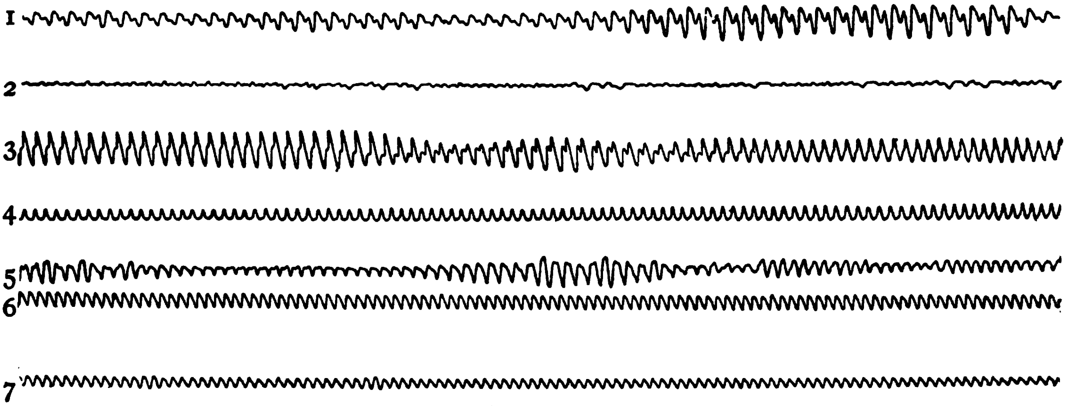

FIG. 2.

For these reasons, a double wave caused by the breaking up of an originally unitary impulse moves slower than did the parent wave. Generally speaking, when a ring is first set into pulsation by electrical stimulation, there are a number of secondary wavelets, but these soon fuse into a single simple wave, and this unitary wave then rarely breaks into component parts.

In *Cassiopea*, as in *Limulus*, the stimulus which produces pulsation is neurogenic, the muscles being incapable of spontaneous pulsation if the nervous system be eliminated. Thus a 0.01 molecular solution of oxalic acid in sea-water is extremely toxic to the motor centers and nerves of *Cassiopea*, a very short immersion preventing the rhopalia from ever again giving rise to the pulsation-stimulus. The muscles, however, still remain capable of contracting when stimulated directly.

In this connection, Harvey found that in most of the amines and in inorganic hydroxides the nerves cease to conduct before the muscles lose their power of contractibility, and under these conditions the muscles respond only when directly stimulated. If the subumbrella tissue be heated, however, the nerves continue to function for several degrees beyond the point whereat the muscles cease to respond by contractions.

The subject of the neurogenic nature of the pulsation-stimulus in *Cassiopea* is discussed by Mayer (1906, p. 19); and, as is well known, Carlson has presented convincing proof that the pulsation-stimulus in the heart of *Limulus* is neurogenic.

The neurogenic nature of pulsation in these invertebrates is the more remarkable because recent work has decidedly strengthened the myogenic theory for the beating of the vertebrate heart. Thus Paton (1907) shows that both the heart and the axial muscular system of *Pristiurus* functions spontaneously before the development of definite nerve-fibrils from the central nervous system, although protoplasmic connectives are seen extending from cell to cell, and these may transmit impulses.

Also, Hooker (1911) finds that the heart differentiates and functions in a normal manner in frog embryos which have developed without a central nervous system; and a crucial experiment has been made by Burrows (1912), who observed that single, isolated muscle-cells of chick hearts, if placed in proper media, pulsate as does the heart itself.

As the experiments of which we are about to speak involve alterations in the composition of the sea-water, it is important that we should understand the composition of the Tortugas sea-water in respect to the cations of sodium, magnesium, calcium, and potassium. Accordingly the following analysis of such of its components as may affect the experiments is presented.

COMPOSITION OF TORTUGAS SEA-WATER.

As a deduction from George Steiger's analysis,[1] made under the direction of Professor F. W. Clarke, it appears that in so far as its ingredients (sodium, magnesium, calcium, and potassium) are concerned, we may make up Tortugas sea-water by mixing the solutions shown under A, each being practically isotonic with the others, and with the sea-water as a whole:

A.					B.		
81.1	volumes of	0.6006	molecular	NaCl.	0.487	molecular	NaCl.
14.36	" "	0.398	"	$MgCl_2$.	0.0571	"	$MgCl_2$.
2.84	" "	0.389	"	$CaCl_2$.	0.0110	"	$CaCl_2$.
1.7	" "	0.597	"	KCl.	0.0101	"	KCl.
100.00							

Thus any given volume of Tortugas sea-water may be considered to consist of an atmosphere composed as in B, all occupying one and the same space.

At 25° C., however, this being near the usual temperature of the tropical sea-water, the NaCl is dissociated to about 75 per cent; and this is also true of approximately 80 per cent of the $MgCl_2$, 88 per cent of the $CaCl_2$, and 95 per cent of the KCl.

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[1] Carn. Inst., Year Book, No. 9, 1910, p. 122.

Thus among every 4,870 molecules of $NaCl$, about 1,217 are at any one instant undissociated, while 3,653 are dissociated; or, expressed in tabular form for the sea-water as a whole:

4,870 molecules of	$NaCl$	= 1,217 fixed	+	3,653 dissociated.
571 "	$MgCl_2$	= 114	+	457
110 "	$CaCl_2$	= 13	+	97
101 "	KCl	= 5	+	96

The $NaCl$ and KCl dissociate each into 2 ions, while the $MgCl_2$ and $CaCl_2$ break up into 3 each, thus:

3,653 dissociated molecules of	$NaCl$ give	3,653 $\dot{Na}$ and	3,653 Cl'.
457 " "	$MgCl_2$	457 $\ddot{Mg}$ "	914 Cl'.
97 " "	$CaCl_2$	97 $\ddot{Ca}$ "	194 Cl'.
96 " "	KCl	96 $\dot{K}$ "	96 Cl'.
Total		4,303	4,857

In other words, in Tortugas sea-water for every 1,000 $\dot{Na}$ cations there are 125 $\ddot{Mg}$, 26 $\ddot{Ca}$, 26 $\dot{K}$; and the anions may be represented by 1,330 Cl'.

As is well known, at least one-fifth of the Cl' anions may be replaced by the SO_4'' ion without appreciable effect, this being accomplished when the sea-water is made up of sulphates of magnesium, calcium, and potassium instead of chlorides. The cations, on the other hand, play a far more definite rôle in the control of movements; for sustained pulsation is impossible in the absence of even the least concentrated cations, such as $\ddot{Ca}$ or $\dot{K}$.

If the sea-water be concentrated to 1.69, by evaporating 100 volumes of natural sea-water to 59 volumes, the molecular concentrations of the various constituents becomes:

0.823	molecular	$NaCl$.
0.0965	"	$MgCl_2$.
0.0186	"	$CaCl_2$.
0.0171	"	KCl.

This results in a relative preponderance of the $\ddot{Mg}$ ions, for the degree of dissociation of the $NaCl$ declines in a greater ratio than that of the $MgCl_2$; for in this concentrated sea-water about 70 per cent of the $NaCl$ is dissociated, as are also about 77 per cent of the $MgCl_2$, 86 per cent of the $CaCl_2$, and 94 per cent of the KCl. Thus:

8,230 molecules of	$NaCl$ give	2,469 fixed and	5,761 dissociated.
965 " "	$MgCl_2$ "	222 " "	743 "
186 " "	$CaCl_2$ "	28 " "	158 "
171 " "	KCl "	10 " "	161 "

And of the dissociated molecules:

5,761 dissociated molecules of	$NaCl$ give	5,761 $\dot{Na}$ and	5,761 Cl'.
743 " " "	$MgCl_2$ "	743 $\ddot{Mg}$ "	1,486 Cl'.
158 " " "	$CaCl_2$ "	158 $\ddot{Ca}$ "	316 Cl'.
161 " " "	KCl "	161 $\dot{K}$ "	161 Cl'.
Total		6,823	7,724

Hence, we see that when the molecular concentration of the sodium, magnesium, calcium, and potassium is 1.69 times that of natural sea-water, the concentration of the cations of these salts is only $\frac{6823}{4303}$ or 1.585, and, therefore, the concentration of the cations has not quite kept pace with that of the sea-water as a whole.

Moreover, in this concentrated sea-water, for every 1,000 $\dot{Na}$ cations there are 129 $\ddot{Mg}$, 27 $\ddot{Ca}$, 28 $\dot{K}$, and 1,340 Cl' anions. Thus the proportionate concentration of Nȧ has decreased with respect to the other cations, and as Nȧ is the most potent stimulant while Mg̈ is the depressant, we might expect the stimulating power of this concentrated sea-water to have increased less rapidly than the ratio of concentration, for in natural sea-water the ratio of the Mg̈ to Nȧ is as 1 to 8, and in sea-water concentrated to 1.69 it is as 1 to 7.75.

If, on the other hand, the sea-water be diluted to 50 per cent of its natural concentration by mixing it with an equal volume of distilled water, a calculation, similar to the one given in detail above, indicates that while the molecular concentration of the dissolved salts has declined to 0.5 (that of natural sea-water being 1), the concentration of the cations is 0.528; and for every 100 Nȧ cations there are 124 Mg̈, 26 Cä, and 25 K̇ cations, and 1,376 Cl′ anions. The results of these several calculations are summarized in table 2.

TABLE 2.

Composition of solution. s. w. = sea-water. d. w. = distilled water.	Gram-molecular concentration of the inorganic salts.				Degree of dissociation.			
	$NaCl$.	$MgCl_2$	$CaCl_2$.	KCl.	$NaCl$.	$MgCl_2$.	$CaCl_2$.	KCl.
					per cent	per cent	per cent	per cent
100 c.c. s. w. + 100 c.c. of d. w.	0.243	0.0285	0.0055	0.005	79.6	84	92	96
Natural sea-water	.487	.0571	.0110	.0101	75	80	88	95
100 volumes of natural sea-water evaporated to 59 volumes	.823	.0965	.0186	.0171	70	77	86	94

Composition of solution. s. w. = sea-water. d. w. = distilled water.	Approximate number of Mg̈, Cä, and K̇ cations for every 1,000 Na cations.			Relative concentration of cations of sea-water.	Relative concentration of sea-water as a whole.
	Mg̈.	Cä.	K̇.		
100 c.c. s. w. + 100 c.c. of d. w.	124	26	25	0.528	0.5
Natural sea-water	125	26	26	1.000	1.0
100 volumes of natural sea-water evaporated to 59 volumes	129	27	28	1.585	1.69

As the osmotic pressure of these solutions is proportional to the respective sums of their fixed molecules and dissociated ions, we find that if the osmotic pressure of Tortugas sea-water be 1, that of 50 per cent sea-water is 0.5004, and that of 1.69 sea-water is 1.64. Thus, knowing that the osmotic pressure of Tortugas sea-water is about 24.8 atmospheres, that of sea-water diluted with an equal volume of distilled water is 12.4, and that of 1.69 sea-water is 40.7 atmospheres. Table 3 shows the relations between the osmotic pressures and concentrations of the various solutions experimented with in this research, and also the molecular concentration of a solution of NaCl which is practically isotonic with each solution. We must remember, however, that pure NaCl solutions, such as those mentioned in this table, are not, strictly speaking, comparable with those found in sea-water. Thus Osterhout (1911) shows that a pure NaCl solution penetrates the membranes of plant-cells more rapidly than if the NaCl is mixed with $CaCl_2$ in the proportions found in sea-water. There is also other evidence of a chemical combination involving the NaCl and $CaCl_2$ of sea-water in their mutual influence upon animals. For example, Mayer (1906, p. 49) states that in the pulsation of *Cassiopea* the calcium of the sea-water assists the NaCl to counteract the inhibiting influence of magnesium, and later Meltzer and Auer, in an important series of studies upon vertebrates, found that solutions containing *both* calcium and sodium counteracted the inhibiting tendencies of magnesium. Experiments upon *Cassiopea* show that calcium alone can not produce tetanus and does not offset the effects of magnesium if sodium be absent, and thus it appears that the so-called "calcium tetanus" is actually a result of the association of sodium with calcium. Mines (1911 A) believes that calcium enters into combination with cardiac tissue, but magnesium does not, and our results support this view.

Fortunately for physiological experimentation, however, *Cassiopea* is but little affected by considerable changes in osmotic pressure, and table 3 is valid for all practical purposes.

TABLE 3.—*Changes in osmotic pressure and in concentration of dissolved salts in diluted and in concentrated sea-water from Tortugas, Florida.*

Composition of solution. s. w. = sea-water. d. w. = distilled water.	Relative concentration, that of sea-water being 1.	Osmotic pressure in atmospheres.	Gram-molecular concentration of an isotonic solution of pure NaCl.	Gram-molecular concentration of NaCl, $MgCl_2$, $CaCl_2$, and KCl of sea-water solution, regarding sea-water as being a solution of chlorides.			
				NaCl.	$MgCl_2$.	$CaCl_2$.	KCl.
35 c.c. s. w. + 65 c.c. d. w.	0.35	8.7	0.206	0.170	0.02	0.0038	0.0035
40 60	.4	9.9	.236	.2	.023	.0044	.004
50 50	.5	12.4	.295	.243	.028	.0055	.005
58 42	.58	14.4	.342	.285	.033	.0064	.0058
60 40	.6	14.9	.354	.295	.034	.0066	.006
66.6 33.3	.66	16.35	.393	.325	.036	.007	.0066
75 25	.75	18.6	.442	.375	.043	.00825	.0075
Pure sea-water	1	24.8	.59	.487	.0571	.011	.0101
100 c.c. s. w. evap'd to 89.3	1.119	27.4	.66	.545	.064	.0123	.0102
86.8	1.152	28.3	.68	.56	.066	.0126	.0115
84.3	1.186	29.2	.70	.58	.068	.013	.0118
80	1.25	30.6	.737	.61	.071	.0137	.0125
78.1	1.28	31.3	.75	.62	.073	.014	.0128
75.7	1.322	32.2	.78	.64	.075	.0145	.0132
71.1	1.407	34.2	.83	.68	.08	.0154	.014
69.2	1.444	35	.85	.7	.082	.0159	.0147
62.5	1.6	38.6	.94	.78	.091	.0175	.016
59.5	1.68	40.5	.99	.819	.095	.0185	.0168
59	1.69	40.7	1.00	.823	.096	.0186	.0169

RELATION BETWEEN THE CONCENTRATION OF THE CATIONS OF SEA-WATER AND THE RATE OF NERVE-CONDUCTION.

If we dilute sea-water by mixing it with distilled water, or concentrate it by slow evaporation at ordinary temperatures, we not only decrease or increase the concentrations of the electrolytes, but we change the osmotic pressure in a nearly equal ratio. We may, however, avoid lowered osmotic changes by diluting the sea-water with a 0.9 molecular solution of pure dextrose,[1] which is practically isotonic with sea-water, and in this manner we may reduce the concentrations of the electrolytes and at the same time maintain the normal osmotic pressure. Thus we may determine the effect of changes in concentration of the electrolytes independent of osmotic pressure.

The results of such experiments are shown in tables 4, 5, and 6, while table 7 shows the arithmetical averages derived from the results recorded in tables 4 to 6. In all these tables the rate is reduced to 100 per minute in natural sea-water in order to facilitate comparisons. Thus, if on the average the medusæ actually pulsated at the rate of 80 per minute in natural sea-water, and at 40 per minute in some other solution, these numbers would become 100 and 50, respectively, in the tables.

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[1] The dextrose used in these experiments was the best that could be obtained from Merck and from Kahlbaum. The accepted samples were tested for sodium and free acids and found to contain inappreciable quantities of these impurities. The dextrose solutions were made up afresh each day.

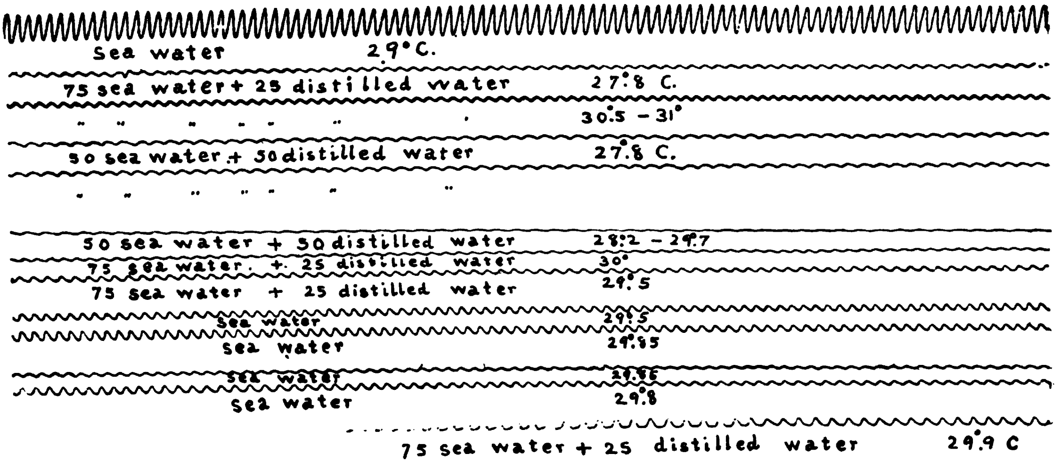

FIG. 3.

Table 4 shows the relative rates of nerve-conduction in 20 rings, and of pulsation in 4 normal medusæ of *Cassiopea xamachana* in sea-water and sea-water diluted with distilled water at 29° to 31° C.

TABLE 4.

Composition of the solution. s. w. = sea-water. d. w. = distilled water.	Osmotic pressure in atmospheres.	Relative rates of pulsation, the rate in normal sea-water being 100.								
		Ring No. 1.[1]	Ring No. 2.	Ring No. 3.	Ring No 4.	Ring No. 5.	Ring No. 6.	Ring No. 7.	Ring No. 8.	Ring No. 9.
Natural sea-water........	24.8	100	100	100	100	100	100	100	100	100
95 p. ct. s. w. + 5 p. ct. d. w.	23.6	......	......	......	......	....	....	....	....	....
90 10	22.3	......	......	......	......	....	....	....	....	....
85 15	21.8	......	......	......	......	....	....	....	....	....
80 20	20.6	......	......	......	......	....	....	....	....	....
75 25	18.6	87	86	88	86	81	86	77	86	89
70 30	17.4	......	......	......	......	....	....	....	....	....
60 40	14.4	......	......	78	......	65	58	....	....	81
50 50	12.4	67	......	64	......	....	....	....	....	62
40 60	9.9	......	......	......	......	....	....	....	....	....
35 65	8.7	......	......	......	......	....	....	....	....	....

Composition of the solution. s. w. = sea-water. d. w. = distilled water.	Osmotic pressure in atmospheres.	Relative rates of pulsation, the rate in normal sea-water being 100.								
		Ring No. 10.	Ring No. 11.	Ring No. 12.	Ring No. 13.	Ring No. 14.	Ring No. 15.	Ring No. 16.	Ring No. 17.	Ring No. 18.
Natural sea-water.........	24.8	100	100	100	100	100	100	100	100	100
95 p. ct. s. w. + 5 p. ct. d. w.	23.6	......	......	......	113	....	105	108	107.5	101
90 10	22.3	......	......	......	121	....	101	113	106	105
85 15	21.8	......	......	......	104	....	99	112	107	105
80 20	20.6	......	......	......	111	....	94	104	96.5	108
75 25	18.6	92	......	94	......	84	....	....	....	....
70 30	17.4	......	......	......	87	....	81	83	77	105
60 40	14.4	50	64	......	......	80	72	74.5	69	93
50 50	12.4	......	50	64	......	....	53	70	61.5	76
40 60	9.9	......	......	......	......	....	....	....	....	....
35 65	8.7	......	......	......	......	....	....	....	....	....

[1] See record, fig. 3

TABLE 4.—*Continued.*

Composition of the solution. s. w. = sea-water. d. w. = distilled water.		Osmotic pressure in atmospheres.	Relative rates of pulsation, the rate in normal sea-water being 100.							
			Ring No. 19.	Ring No. 20.	General average for the 20 rings.	Normal medusa No. I.	Normal medusa No. II.	Normal medusa No. III.	Normal medusa No. IV.	Normal medusa No. V.
Natural sea-water.........		24.8	100	100	100	100	100	100	100	100
95 p. ct. s. w. +	5 p. ct. d. w.	23.6	103	82	103	......	......	......	......	......
90	10	22.3	100	97	105	......	......	......	......	......
85	15	21.8	89	99	101	......	......	......	......	......
80	20	20.6	84	106	100.5	......	......	......	......	......
75	25	18.6	......	......	86	33	57	......	30	21
70	30	17.4	75	70	82.5	......	......	......	......	......
60	40	14.4	67.5	62	70	......	......	90	30	16
50	50	12.4	53	50.5	61	12	13	60	30	13
40	60	9.9	......	......	......	......	7	40	7	......
35	65	8.7	......	......	......	......	......	28	3	......

TABLE 5.

Composition of the solution. s. w. = sea-water.		Relative rates (rate in pure sea-water being 100).							
		Ring No. 1.[1]	Ring No. 2.	Ring No. 3.	Ring No. 4.	Ring No. 5.	Ring No. 6.	Ring No. 7.	Ring No. 8.
Natural sea-water, salinity 36.15 p. ct.		100	100	100	100	100	100	100	100
95 p. ct. s. w. +	5 p. ct. dextrose.....	......	......	......	......	......	......	......	......
90	10	......	......	......	......	......	......	......	......
85	15	......	......	......	......	......	......	......	......
80	20	......	......	......	......	......	......	......	......
75	25	86	......	86	86	92	98	96	98
71	29	......	......	......	......	......	......	......	......
66.6	33.3	......	......	......	......	......	......	......	......
60	40	......	80	76.5	73	78	73	89	70
58	42	......	......	......	......	......	......	......	......
50	50	55.5	63	......	......	63	......	75	51

Composition of the solution. s. w. = sea-water.		Relative rates (rate in pure sea-water being 100).							
		Ring No. 9.	Ring No. 10.	Ring No. 11.	Ring No. 12.	Ring No. 13.	Ring No. 14.	Ring No. 15.	Ring No. 16.
Natural sea-water, salinity 36.15 p. ct..		100	100	100	100	100	100	100	100
95 p. ct. s. w. +	5 p. ct. dextrose.....	......	101	......	......	......	103	......	......
90	10	......	......	104	105	102	101	......	......
85	15	......	......	......	......	......	......	101	103
80	20	......	98	......	......	......	......	100.5	97.5
75	25	96	95	......	......	......	......	......	......
71	29		......	82	......	86	92	......	......
66.6	33.3	......	......	......	......	......	......	......	......
60	40	59	79	......	......	......	......	69	......
58	42	......	......	......	......	......	......	......	......
50	50	47	......	55	......	47	......	......	......

Composition of the solution. s. w. = sea-water.		Relative rates (rate in pure sea-water being 100).						
		Ring No. 17.	Ring No. 18.	Ring No. 19.	Ring No. 20.	Gen. av. 20 rings.	Normal medusa No. I.[2]	Normal medusa No. II.[3]
Natural sea-water, salinity 36.15 p. ct..........................		100	100	100	100	100	100	100
95 p. ct. s. w. +	5 p. ct. dextrose.	......	105	114	96	104	......	......
90	10	......	115	109	......	106	......	......
85	15	103	......	......	108	104	......	......
80	20	99	105	100	......	100	......	......
75	25	......	......	......	......	92.5	47	......
71	29	......	......	......	......	87	......	......
66.6	33.3	......	......	......	......	......	......	23
60	40	......	......	......	66.5	73	......	......
58	42	......	......	......	......	......	......	2
50	50	......	50	57	58	56.5		

[1] See record, fig. 4.
[2] Six hours after being restored to sea-water, rate was nearly normal with amplitude lower than normal
[3] When restored to sea-water, this medusa pulsated at about two-thirds normal rate. See record, fig. 5

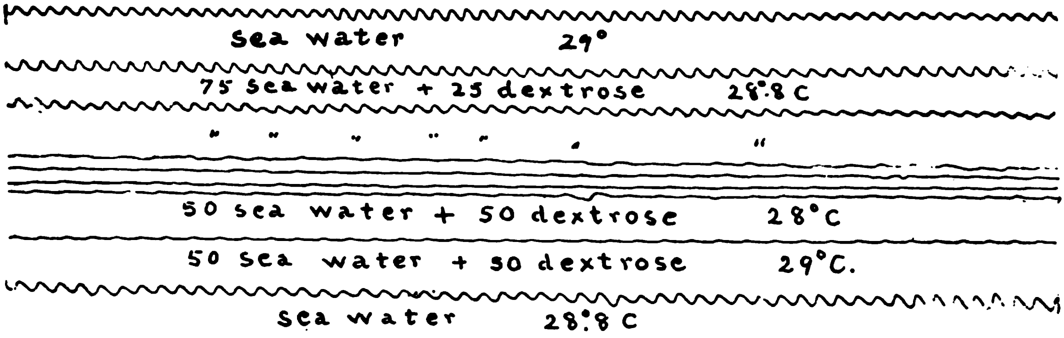

FIG. 4.

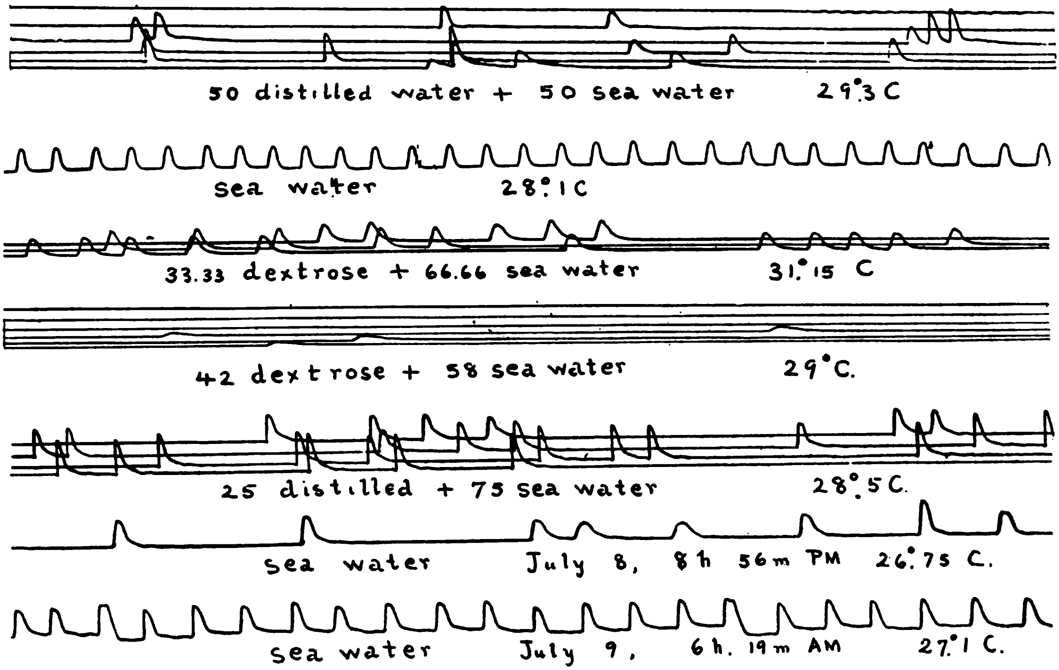

FIG. 5.

Table 5 shows the relative rates of pulsation of 2 medusæ and of nerve-conduction in 20 rings of subumbrella tissue of *Cassiopea* in sea-water, and in sea-water mixed with 0.9 molecular dextrose.

Table 6[1] shows the relative rates of nerve-conduction in subumbrella tissue of *Cassiopea xamachana* in sea-water diluted with distilled water, and also in sea-water diluted with 0.9 molecular dextrose. (See tables 4 and 5.) The practical coincidence of these two curves indicates that the changes in rate of nerve-conduction are due to corresponding changes in the concentration of the electrolytes and not to changes in osmotic pressure.

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[1] See fig. 6, which shows that these two curves are apparently identical, the differences between them being probably due to errors of experimental nature and individual variation.

In figure 6 the full line and open circles represent the rates in sea-water diluted with 0.9 molecular dextrose, and the dotted line and black circles show the rates in sea-water diluted with distilled water.

TABLE 6.

Composition of the solution. s. w. = sea-water. d. w. = distilled water.	Relative rates of nerve-conduction, that in natural sea-water being 100.	
	Sea-water diluted with distilled water, thus correspondingly reducing osmotic pressure.	Sea-water diluted with 0.9 molecular dextrose, thus maintaining normal osmotic pressure of 24.8 atmospheres.
Natural sea-water, salinity 36.15 p. ct.....	100	100
95 p. ct. s. w. + 5 p. ct. d. w. or dextrose...	103	104
90 10 ..	105	106
85 15 ..	101	104
80 20 ..	100.5	100
75 25 ..	86	92.5
71 29 ..	....	87
70 30 ..	82.5	....
60 40 ..	70	73
50 50 ..	50	56.5

Figure 6 gives a graphic representation of the results stated in table 6, the ordinates giving the average rates of the pulsating rings, and the abscissæ giving the relative concentration of the dissolved salts of the sea-water, that of normal sea-water being 100. Thus the ordinates of the full-line curve show the relative rates of conduction of the nerve-impulse when the sea-water is diluted with 0.9 molecular dextrose, and the dotted curve shows the same for sea-water diluted with distilled water so as to reduce its osmotic pressure in nearly the same ratio as the reduced concentration of the electrolytes. The practical coincidence of the curves for sea-water, diluted with distilled water and with 0.9 molecular dextrose, shows that the phenomena in question are not due to variations in osmotic pressure, but to the varying concentrations of the electrolytes.

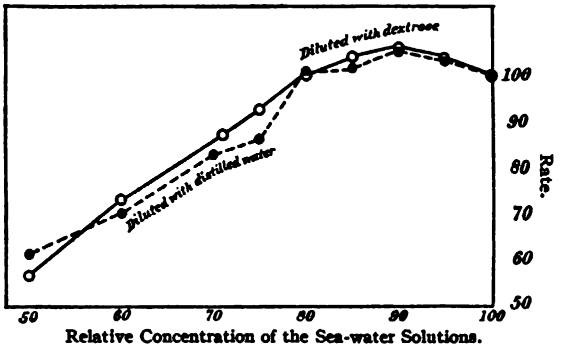

FIG. 6.

Moreover, both these curves show that the most rapid rate of nerve-conduction is in diluted sea-water, wherein the electrolytes are only about

90 per cent as concentrated as in natural sea-water. Below this the rate declines, becoming normal in 80 per cent sea-water and from this point downward it declines in a straight-line ratio, at a somewhat more rapid rate than the corresponding decline in concentration of the dissociated electrolytes.

The curve as a whole reminds one of that of an enzyme reaction having its maximum in 90 per cent sea-water and rising in a straight line from 50 to 80 per cent. This does not *prove*, however, that it has anything to do with enzyme action.

It will be recalled that Loeb (1891) discovered that growth and regeneration in hydroids proceeds at a somewhat more rapid rate in sea-water diluted with 15 to 20 per cent distilled water than in normal sea-water. The experiments of Goldfarb (1907) upon the regeneration and growth rate in hydroids and his more recent observations upon *Cassiopea*[1] confirm this fact. Loeb ascribed the increase in rate of regeneration to the effects of potassium and magnesium, but Goldfarb's observations cast doubt upon this simple explanation. It is clear that the initial increase in the rate of nerve-conduction in *Cassiopea*, which accompanies the decline in concentration of the electrolytes below the normal, is not due to lowered osmotic pressure, for it occurs when the sea-water is diluted with 0.9 molecular dextrose, thus maintaining the osmotic pressure found in normal sea-water.

A possible but purely hypothetical explanation may be that if the rate of nerve-conduction is due to an excess of ionic sodium in the tissues over and above its concentration in the surrounding sea-water, a slight lowering of the concentration of the sodium in the sea-water causes a corresponding increase in its ratio of concentration within the tissues and may thus cause an augmentation in the stimulus. There is some evidence to support this view, for when the sea-water is diluted with 0.487 molecular sodium chloride, the rate does not increase as rapidly as it does when the sea-water is diluted with distilled water or with dextrose. The slight initial increase in rate when the sea-water is diluted with 0.487 molecular NaCl may be due to the reduction of the potassium ion which in such concentration is a stimulant, as is sodium.

THE RELATION BETWEEN THE RATE OF NERVE-CONDUCTION AND THE CONCENTRATION OF THE SODIUM ION.

In order to test the effect of the sodium cation upon the rate of nerve-transmission, we may add increments of a solution of 0.6 molecular NaCl to the sea-water, thus maintaining a constant and normal osmotic pressure, but increasing the concentration of Nä while the concentrations of Mg̈, Cä, and K̇ are decreased.

The physical effects of such a procedure are illustrated in table 7, showing that if we add 0.6 molecular NaCl up to 100 sea-water + 800 NaCl we have changed the concentration of the sodium ion from 1 to 1.205; while at the same time, in comparison with the Mg̈, Cä, and K̇, the Nȧ ion has become relatively 10.85 times as concentrated as it was in natural sea-water.

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[1] See Dr. Goldfarb's paper upon the rate of regeneration of *Cassiopea* in concentrated, normal, and diluted sea-water; page 88 of this volume.

TABLE 7.

Composition of the solution. s. w. = sea-water.	Relative dilution of the Mg̈, Cä, and K̇ of sea-water.	Relative concentration of Nä of sea-water.	Proportionate concentration of Nȧ compared with that of the Mg̈, Cä, and K̇ (proportion in sea-water being 1.00).	Gram-molecular concentrations of—			
				NaCl.	MgCl₂.	CaCl₂.	KCl.
Natural sea-water.....	1.00	1.00	1.00	0.487	0.057	0.0110	0.0101
100 s. w. + 10 NaCl....	.909	1.008	1.1	.491	.052	.0099	.0091
100 20	.833	1.03	1.23	.506	.047	.00916	.0084
100 30	.7615	1.04	1.4	.51	.043	.00837	.0077
100 40	.714	1.06	1.48	.52	.0407	.00785	.0072
100 50	.666	1.07	1.6	.525	.038	.00739	.0067
100 60	.625	1.08	1.7	.529	.0356	.00687	.0063
100 70	.588	1.09	1.85	.533	.034	.0064	.0059
100 80	.555	1.1	1.98	.537	.0316	.0061	.0055
100 90	.526	1.108	2.1	.54	.03	.0058	.0053
100 100	.50	1.11	2.22	.543	.0285	.0055	.005
100 200	.333	1.15	3.45	.562	.019	.0036	.0033
100 700	.125	1.203	9.62	.586	.007	.0013	.0012
100 800	.111	1.205	10.85	.587	.006	.0012	.0011

Table 8 shows the relative rates of nerve-conduction in four rings of *Cassiopea xamachana* in sea-water to which increments of 0.6 molecular NaCl are added.

TABLE 8.

Composition of the sea-water. s. w. = sea-water.	Relative concentration of Cä, Mg̈, and K̇ of sea-water.	Ring No. 1.	Ring No. 2.	Ring No. 3.	Ring No. 4.	Average condition reduced to 100 pulsations per minute in natural sea-water.
Natural sea-water......	1.00	100	100	100	100	[1]100
100 s. w. + 10 NaCl....	.909	103	....	....	....	103
100 20	.833	102	....	....	....	102
100 30	.7615	105	....	....	....	105
100 40	.714	108	....	....	....	108
100 50	.666	96	....	....	....	96
100 60	.625	97	....	....	....	97
100 70	.588	102	....	....	....	102
100 80	.555	87	....	....	....	87
100 90	.526	82	....	....	....	82
100 100	.50	101	85	....	....	93
100 200	.333	....	90	....	....	90
100 700	.125	....	....	66	87	76.5

[1] See fig. 7, dotted curve with determined points marked by open circles.

Figure 7 shows the stimulating influence of sodium. The dotted curve with determined points marked by open circles represents the effect of 0.6 molecular sodium chloride. The full-line curve shows the less marked effect of 0.487 molecular sodium chloride, and the dextrose curve is introduced for purposes of comparison.

The average results are plotted in the upper dotted line in figure 7 and it appears that the rate of the ring increases slowly in proportion as the sodium becomes concentrated relatively to the other cations, until the relative concentration of the sodium in respect to the other cations is about 1.48 times that found in natural sea-water. At still greater concentration of sodium the rate declines slowly, but not so rapidly as it does in sea-water diluted with a 0.9 molecular solution of dextrose, thus showing the stimulating influence of the sodium ion.

By diluting the sea-water with 0.9 molecular dextrose, we maintain a constant and normal osmotic pressure, but all the cations are reduced in concentration, as they would have been had we added distilled water. On the other hand, when we add 0.6 molecular NaCl we increase the absolute concentration of the sodium, while the osmotic pressure of the solutions as a whole remains constant, but the chief effect is to increase the relative concentration of the sodium cation with respect to magnesium, calcium, and potassium.

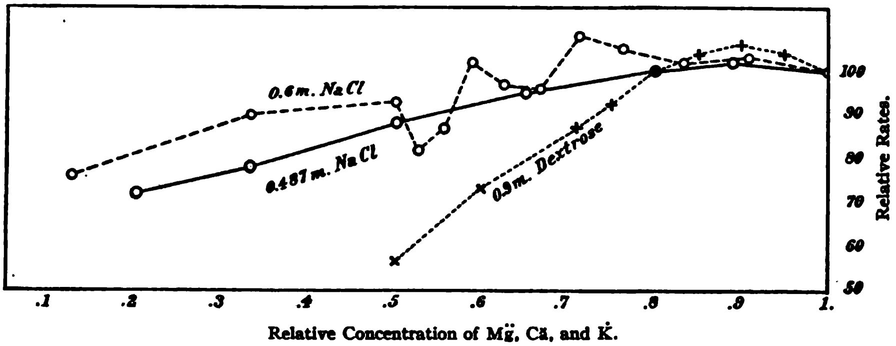

FIG. 7.

We may conclude that sodium is a powerful stimulant up to about 1.48 times its relative concentration with respect to the other cations in natural sea-water augmenting the rate of nerve-conduction in proportion as its relative concentration increases. In concentrations over and above 1.48 times that found in sea-water, it loses some of its stimulating effect, but the rate of pulsation of the ring declines more slowly than it would in sea-water diluted with a 0.9 molecular solution of dextrose, and thus the presence of the sodium still acts as a constant stimulant.

The experiment cited above is open to the serious objection that the addition of 0.6 molecular NaCl increases the concentration of the Nȧ ion over and above that found in natural sea-water, and the stimulating effect is evidently due in part at least to this increase, and not to the proportionate decrease in concentration of magnesium, calcium, and potassium. In order to avoid this error, we should add the NaCl in the form of a 0.487 molecular solution dissolved in a 0.075 molecular solution of dextrose, thus maintaining the normal osmotic pressure and the normal concentration of the sodium ion—*simply reducing* the concentrations of the magnesium, calcium, and potassium, and correspondingly increasing the relative concentration of the sodium cation.

Table 9 shows the results of this experiment upon 20 subumbrella rings of *Cassiopea*, and here again it is evident that the sodium cation is a stimulant to nerve-conduction. It maintains the rate of nerve-conduction at about a normal rate until the magnesium, calcium, and potassium are reduced to 80 per cent of the concentration found in natural sea-water.

In still greater dilutions there is a slow decline in rate in a rectilinear ratio, but the decline is not so rapid as it is when all four cations, sodium, magnesium, calcium, and potassium, are reduced by adding 0.9 molecular dextrose to the sea-water. Thus the sodium of the normal sea-water is a stimulant to the rate of nerve-conduction.

As we would expect, the 0.6 molecular NaCl is more stimulating than the lower concentration, 0.487 molecular.

TABLE 9.

Composition of solution. s. w. = sea-water.	Relative rates of pulsation, the rate in normal sea-water being 100.						
	Ring No. 1.	Ring No. 2.	Ring No. 3.	Ring No. 4.	Ring No. 5.	Ring No. 6.	Ring No. 7.
Sea-water (salinity 36.15 p. ct., osmotic pressure 24.8 atmospheres)........	100	100	100	100	100	100	100
89 p. ct. s. w. + 11 p. ct. (NaCl + dextrose)....	......	......	......	......	......	......	103
80 20	......	......	......	......	102	98	101
65 35	......	......	......	......	......	......	......
50 50	87.5	86	85.5	97	82	84	......
33.3 66.6	......	......	77.5	78	74	......	......
20 80	......	62.5	65.5	85.5	......	......	70.5

Composition of solution. s. w. = sea-water	Relative rates of pulsation, the rate in normal sea-water being 100.						
	Ring No. 8.	Ring No. 9.	Ring No. 10.	Ring No. 11.	Ring No. 12.	Ring No. 13.	Ring No. 14.
Sea-water (salinity 36.15 p. ct., osmotic pressure 24.8 atmospheres)........	100	100	100	100	100	100	100
89 p. ct. s. w. + 11 p. ct. (NaCl + dextrose)....	99	113	99	98	......	......	......
80 20	90.5	120	92	86	102.5	106	102
65 35	......	......	......	......	......	......	......
50 50	......	......	......	......	95	......	90
33.3 66.6	84	78	......	......	......	......	......
20 80	......	......	......	......	......	......	......

Composition of solution. s. w. = sea-water.	Relative rates of pulsation, the rate in normal sea-water being 100.						
	Ring No. 15.	Ring No. 16.	Ring No. 17.	Ring No. 18.	Ring No. 19.	Ring No. 20.	Gen. av.
Sea-water (salinity 36.15 p. ct., osmotic pressure 24.8 atmospheres)........	100	100	100	100	100	100	[1]100
89 p. ct. s. w. + 11 p. ct. (NaCl + dextrose)....	......	......	......	......	......	......	102
80 20	......	......	......	......	......	......	100
65 35	100	81	96	103.5	......	......	95
50 50	......	......	......	......	......	......	88
33.3 66.6	78	......	......	......	68.5	75	78
20 80	......	......	......	......	......	......	72

[1] See fig. 7, full-line curve.

Table 9 shows the relative rates of nerve-conduction in 20 subumbrella rings of *Cassiopea xamachana* in sea-water and in sea-water diluted with a solution composed of 0.487 molecular NaCl dissolved in 0.075 molecular dextrose. This solution maintains the normal concentration of NaCl and the normal osmotic pressure of Tortugas sea-water and its addition merely causes a decline in the concentrations of the magnesium, calcium, and potassium of the sea-water.

As in all other experiments of this series, the perfect medusæ are more sensitive to the effects of sodium than are the rings, but the difference in

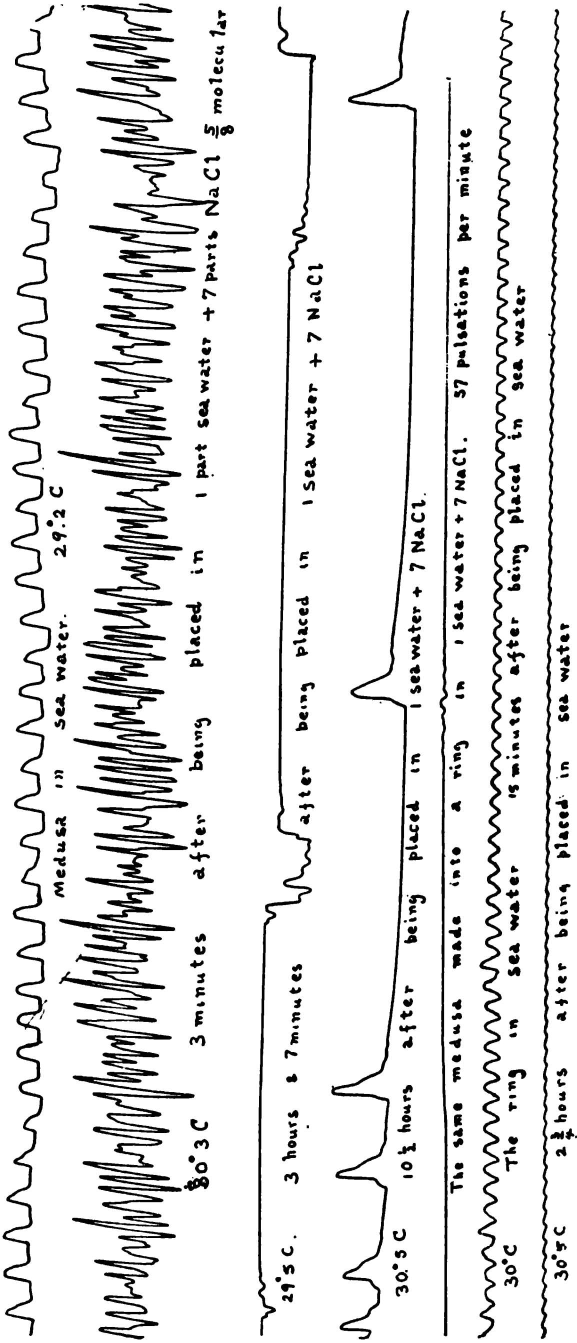
Medusa in sea water. 29.2° C
30°3 C 3 minutes after being placed in 1 part sea water + 7 parts NaCl 5/8 molecular
29°5 C. 3 hours & 7 minutes after being placed in 1 Sea water + 7 NaCl
30°5 C 10½ hours after being placed in 1 sea water + 7 NaCl.
The same medusa made into a ring in 1 sea water + 7 NaCl. 57 pulsations per minute
30°C The ring in sea water 15 minutes after being placed in sea water
30°5 C 2¾ hours after being placed in sea water

FIG. 8.

their behavior is one of degree rather than of kind. In other words, the motor centers are merely a more sensitive part of the nervous system than is the nerve-net of the subumbrella.

For example, a medusa of *Cassiopea xamachana* (record, fig. 8) with motor centers intact, and pulsating at the rate of 33 per minute in natural sea-water, pulsated very irregularly at the rate of about 8 per minute after having been for 10.5 hours in a solution of 100 volumes of sea-water + 700 volumes of 0.6 molecular NaCl. Here we see the decline in pulsation-rate is greater than in the nervous network without motor centers. If, however, the sea-water had been diluted with 0.9 dextrose, the medusa would have ceased to pulsate when the sea-water had been mixed with about an equal volume of dextrose; hence the effect of the lowering of concentration of $\mathrm{M\ddot{g}}$, $\mathrm{C\ddot{a}}$, and $\dot{\mathrm{K}}$ and proportionately increasing the concentration of the sodium ion is to stimulate and maintain pulsation.

The initial effect of an increased concentration of NaCl upon the normal medusa is to increase its rate of pulsation. Thus the medusa which pulsated at the rate of 33 per minute in sea-water, immediately pulsated at the rate of about 116 per minute when placed in 1 volume of sea-water plus 7 volumes of 0.6 molecular NaCl, although after half an hour its rate was about normal and after 10.5 hours it was only about 8 per minute. Thus the initial effect of the relatively increased concentration of sodium was to stimulate, but later it produced depression in a manner resembling the effect of its close chemical ally, potassium. It is interesting to see that Magowan (1908) and Osterhout (1909) find that sodium and potassium are closely similar both in their toxic and protective action upon plants.[1]

THE INERT NATURE OF THE MAGNESIUM ION.

In order to determine the effects of the magnesium ion, we may add magnesium to sea-water in the form of a 0.057 molecular solution of $MgCl_2$ dissolved in 0.78 molecular dextrose. This decreases the concentration of the sodium, calcium, and potassium cations, but maintains the normal concentration of the magnesium ion and also the normal osmotic pressure of the sea-water. The effect must, therefore, be due to the decline in concentration of the sodium, calcium, and potassium.

Table 10 and figure 9 (dotted curve with open circles) show the results of this experiment upon 7 rings of subumbrella tissue of *Cassiopea*, and it indicates that the magnesium ion, in the concentration found in sea-water, is not in any sense a stimulant; for the rate of nerve-conduction declines in practically the same manner is it does when the sea-water is diluted with distilled water or with 0.9 molecular dextrose.

The non-toxic nature of magnesium is clearly shown by the good and rapid recovery of the rate of pulsation when the rings are replaced in natural sea-water.

Figure 9 shows the effects of magnesium on the rate of nerve-conduction. The full-line curve shows the effect of adding 0.4 molecular $MgCl_2$ to sea-water. The dotted curve applies to 0.057 molecular $MgCl_2$. The crosses show the effects of adding 0.9 molecular dextrose to sea-water, and the black circles show the effects of increments of distilled water added to sea-water.

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[1] Botanical Gazette, vol. 45, p. 45; vol. 48, p. 98.

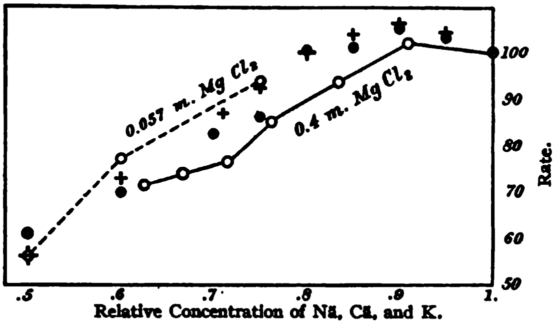

FIG. 9.

Table 10 shows the decline in rate of nerve-conduction in 7 subumbrella rings of *Cassiopea* in sea-water diluted with a solution composed of 0.057 molecular $MgCl_2$ + 0.78 molecular dextrose.

TABLE 10.

Composition of the solution. s. w. = sea-water.	Relative rate of nerve-conduction, the rate in normal sea-water being 100.							
	Ring No. 1.	Ring No. 2.	Ring No. 3.	Ring No. 4.	Ring No. 5.	Ring No. 6.	Ring No. 7.	Gen. av.
Natural sea-water...................	100	100	100	100	100	100	100	100
90 p.ct. s. w. + 10 p.ct. $MgCl_2$ + dextrose[1]	....	....	....	....	....	....	....	....
75 25	97	99	91	97	100	92	90	95
60 40	79	80	82	89	80	79	54	77.5
50 50	....	71	....	56	49.5	55	47.5	56
Rate an hour after being returned to normal sea-water...................	....	91	....	....	93	99	....	....

[1] Only two rings were tested in this solution and they both gave relative rates of 94. This rate is probably too low to be typical, and therefore I have rejected the results obtained from these two rings and omitted them from the table.

By another method, which was tried at Tortugas in 1911 and 1912, the normal osmotic pressure was maintained by adding to the sea-water a 0.4 molecular solution of $MgCl_2$ in distilled water. This causes a dilution of

TABLE 11.

Composition of the solution. n. s. w. = natural sea-water.	Gram-molecular concentration of NaCl, $MgCl_2$, $CaCl_2$, and KCl.				Relative concentration of the Nȧ, Cä, and K̇, their concentration in natural sea-water being 1.	Relative concentration of the Mg̈, their concentration in natural sea-water being 1.	Proportionate concentration of Mg̈ compared with that of Nȧ, Cä, and K̇, the proportion in natural sea-water being 1.
	NaCl.	$MgCl_2$.	CaCl.	KCl.			
Natural sea-water..................	0.487	0.0571	0.0110	0.0101	1.000	1.000	1.0
100 c.c. n. s. w. + 10 c.c. $MgCl_2$.......	.443	.0883	.00999	.00918	.909	1.546	1.7
100 20	.406	.1142	.00916	.00841	.8333	2	2.4
100 30	.3745	.1372	.00837	.0077	.7615	2.402	3.15
100 40	.348	.1550	.00785	.0072	.714	2.714	3.801
100 50	.325	.1713	.00739	.0067	.666	3	4.504
100 60	.304	.1875	.00687	.00631	.625	3.284	5.25

the sodium, calcium, and potassium ions and an augmentation in the concentration of the magnesium, as is shown in table 11.

Table 12 gives the decline in rate of pulsation shown by 2 rings and 9 normal medusæ of *Cassiopea xamachana* in sea-water to which increments of 0.4 molecular $MgCl_2$ have been added.

TABLE 12.

Composition of the solution. s. w. = sea-water.	Relative concentrations of Na, Ca, and K.	Relative rates of pulsation.					
		Ring No. 1.[1]	Ring No. 2.[2]	Medusa No. 3.[3]	Av. of medusæ Nos. 4 and 5.[4]	Av. of medusæ Nos. 6, 7, and 8.[5]	Av. of medusæ Nos. 9, 10, and 11.[6]
Natural sea-water............	1.00	[7]100	100	[8]100	100	100	100
100 c.c. s. w. + 2.5 c.c. $MgCl_2$..	.97	......	......	......	......	......	65.5
100 5 ..	.95	......	......	......	62	70	80
100 7.5 ..	.93	......	......	......	......	......	63
100 10 ..	.909	107	97.5	51	28	51	46
100 12.5 ..	.888	......	......	......	......	......	31
100 15 ..	.871	......	......	......	23	35	19
100 17.5 ..	.85	......	......	......	......	......	21
100 20 ..	.833	94	94	23	15	23	14.15
100 22.5 ..	.817	......	......	......	......	......	6
100 25 ..	.8	......	......	......	11	1	6
100 27.5 ..	.78	......	......	......	......	......	1
100 30 ..	.761	88.5	82	......	0	0	......
100 40 ..	.714	75	78	......	......	......	......
100 50 ..	.666	68	80	......	......	......	......
100 60 ..	.625	71	72	0	......	......	......

[1] 15.5 hours after being replaced in sea-water this ring pulsated at the normal rate.
[2] 3 hours after being replaced in sea-water this ring pulsated at its normal rate.
[3] After being replaced in sea-water this medusa recovered perfectly and pulsated at its normal rate.
[4] After being replaced in sea-water they soon recovered and pulsated normally.
[5] These medusæ recovered completely and pulsated normally after being replaced in sea-water.
[6] Upon replacing these medusæ in sea-water, they pulsated at normal rates.
[7] A partial record of this ring is given in record, fig. 19.
[8] See record, fig. 11.

Table 13, which is derived from table 12, shows the average rates of nerve-conduction in two rings of *Cassiopea xamachana* in sea-water and in sea-water mixed with 0.4 molecular $MgCl_2$.

TABLE 13.

Composition of the solution. s. w. = sea-water.	Av. rate per min. of the two rings.	Av. rate reduced to 100 per min. in normal sea-water.	Dilution of the Na, Ca, and K of the sea-water.	Relative concentration of Mg, its concentration in sea-water being 1.
Natural sea-water..............	141.5	[1]100	1.0	1.0
100 s. w. + 10 $MgCl_2$............	144.5	102	.909	1.7
100 20	133	94	.833	2.4
100 30	120	85	.7615	3.15
100 40	108	76.5	.714	3.8
100 50	104	74	.666	4.5
100 60	100.5	71.5	.625	5.25

[1] See fig. 9, full-line curve.

Table 14 shows the average rates of pulsation of 9 medusæ of *Cassiopea xamachana* in sea-water and in sea-water mixed with 0.4 molecular $MgCl_2$. The rates are reduced to a scale of 100 in natural sea-water.

Tables 12 and 13 show the results of experiments upon two pulsating rings in sea-water at 28° to 31° C. to which had been added increments of 0.4 molecular $MgCl_2$ dissolved in distilled water. The rings or medusæ were permitted to pulsate in any new concentration of magnesium for at least 1 and usually 3 hours before a record of the pulsation-rate was taken, and in the interval the solution was changed several times. In this manner an attempt was made to permit the animal to become thoroughly permeated by a solution before its effect was tested.

TABLE 14.

Composition of the solution. s. w. = sea-water.	Relative rate reduced to 100 in normal sea-water.	Dilution of concentration of $\dot{Na}$, $\ddot{Ca}$, and $\dot{K}$ of the sea-water.	Relative concentration of Mg (concentration in sea-water being 1).
Natural sea-water	100	1.000	1.0
100 c.c. s. w. + 10 $MgCl_2$	44	.909	1.7
100 20	17.5	.8333	2.4
100 25	2.5	.8	2.2
100 30	0	.7615	3.15

Tables 13 and 14 show the average conditions; table 13 referring to the rate of nerve-conduction in the rings without motor centers, and table 14 to the pulsation of medusæ with motor centers intact. It is evident that any excess of magnesium causes a rapid decline in rate in the perfect medusæ.

In rings of subumbrella tissue 0.4 molecular $MgCl_2$ causes a decline in rate of nerve-conduction which is but little more pronounced than that observed when the sea-water is diluted with distilled water or with 0.9 molecular dextrose.

This leads one to conclude that the magnesium ion is inert rather than an active repressor of nerve-conduction. If this be the case all concentrations of $MgCl_2$ from 0.4 molecular downward should behave alike and should exert the same influence as distilled water or 0.9 molecular dextrose.

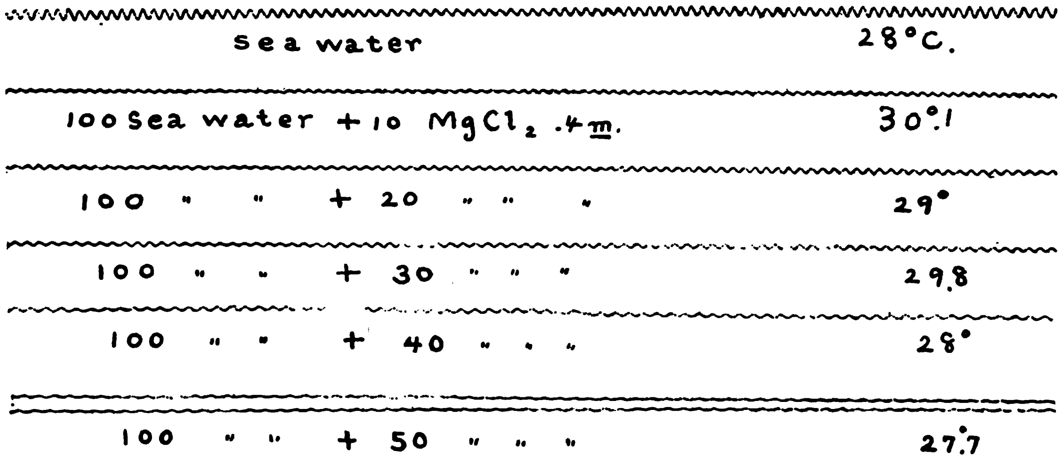

FIG. 10.

Figure 9 appears to show that this expectation is largely realized, the effects of 0.057 molecular and of 0.4 molecular $MgCl_2$ being closely similar to those of distilled water, and while 0.4 molecular $MgCl_2$ may exert a slight specific depressant effect, it is evident that the concentration of $\ddot{Mg}$ found in sea-water (0.057 molecular) is not more depressant than is distilled water.

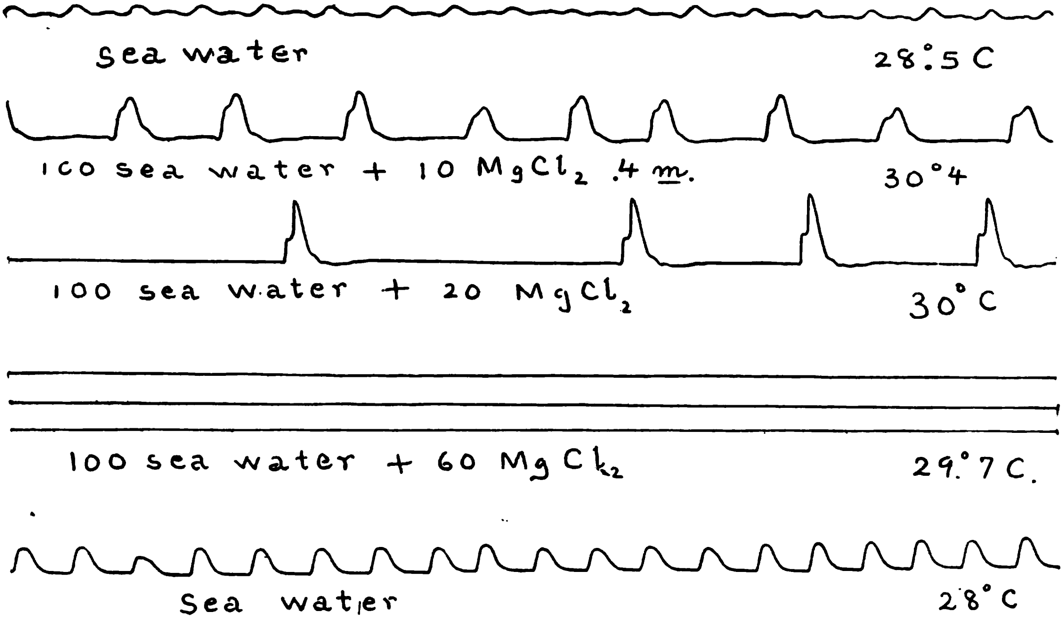

FIG. 11.

Thus apart from osmotic effects the degree of concentration of magnesium makes but little difference in stupefying marine animals by Tullberg's method of adding $MgCl_2$ or $MgSO_4$ to the sea-water. The comparative unimportance of the magnesium ion may also account for the fact that while the relative concentrations of the sodium, calcium, and potassium in the blood of mammals remain pretty much as they are in sea-water, the magnesium has declined from 3.7 to 0.4 or to about one-ninth its former concentration (see "The fitness of the environment," p. 187, by Laurence J. Henderson, New York, 1913).

Normal medusæ soon cease to pulsate if placed in a solution of 100 c.c. sea-water + 30 c.c. of 0.4 molecular $MgCl_2$, and they may remain in this solution for 8 days without further pulsation, the bell, however, slowly disintegrating, due to bacterial action which the weakened concentration of sodium is unable to check. Whenever these magnesiumized medusæ are replaced in normal sea-water they at once go into clonic tetanus and then pulsate normally. This initial tetanus resembles that which develops in sea-water lacking magnesium, and is there due to the combination of sodium and calcium unchecked by magnesium. It seems, then, that an excess of magnesium acts as a shield or "buffer" to prevent the calcium from producing tetanus, and when this shield is suddenly removed the medusa is unable to withstand the effects of even the normal concentration of calcium.[1] It will be recalled that Mines (1911 B, p. 185) arrested the pulsation of the heart of a ray by magnesium and then restored pulsation by simply raising the hydrogen ion concentration of the solution from about 6.5 to 9. Thus the inhibiting effect of the $\ddot{Mg}$ was offset by the addition of an alkali.

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[1] Sörensen, 1909, Comptes Rendus Lab. Carlsberg, vol. 8, p. 1, calls attention to the action of various sorts of "buffer" substances in physiological solutions.

It appears from my experiments of 1906 that calcium can not produce tetanus unless sodium be present, for in the absence of sodium even pure solutions of 0.4 molecular $CaCl_2$ do not produce tetanus in *Cassiopea*, and if the medusæ be stupefied in magnesium they can not be temporarily revived by calcium unless sodium be present. These observations are in accord with those of Meltzer and Auer on mammals, wherein they found that the inhibiting tendency of magnesium is offset by a calcium-sodium combination, not by calcium alone.

As in all experiments of this series, normal medusæ are much more sensitive to the effects of magnesium than are rings. In other words, as is always the case, the motor centers are more readily affected than are the peripheral nerves.

EFFECTS OF THE POTASSIUM ION.

We may maintain the normal osmotic pressure and increase the concentration of the potassium ion by adding to the sea-water a 0.64 molecular solution of KCl. Even so small an amount as 3.75 c.c. of this solution added to 100 c.c. of sea-water stops all movement in normal medusæ after an initial excitement which soon subsides. The peripheral nerves, on the other hand, are not so readily affected as the motor centers, for a ring still pulsated at about two-thirds its normal rate when placed in 100 c.c. sea-water + 7.5 c.c. of 0.64 molecular KCl.

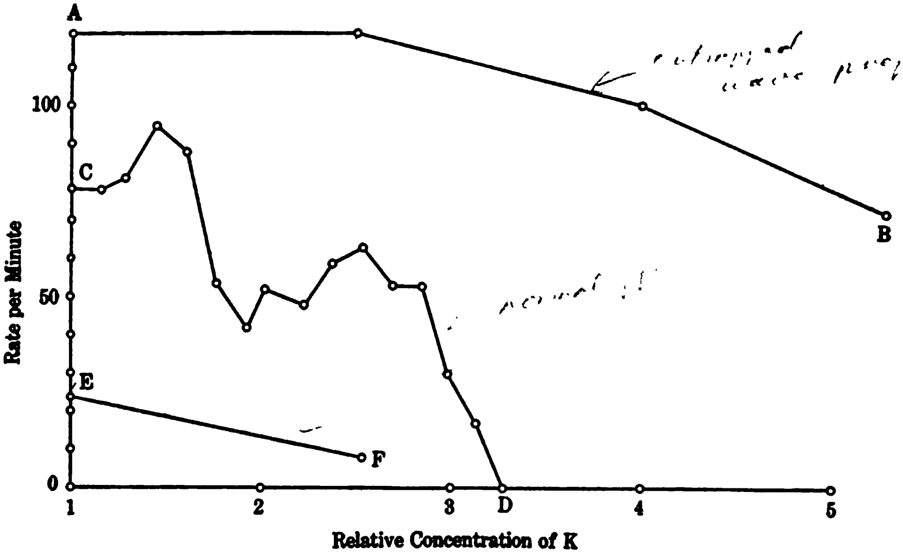

FIG. 12.

Table 15 and figure 12 illustrate the results of experiments with potassium. The rates stated in figure 12 are the actual rates assumed by animals after being at least 45 minutes in the solutions mentioned, the volume of solution being very great in comparison with the size of the animals and the solutions being changed frequently.

It appears that a very slight increase over and above the normal concentration of the potassium ion is a permanent stimulus.

Table 15 shows the relative rates of 3 normal medusæ and 1 subumbrella ring of *Cassiopea xamachana* in sea-water to which increments of a 0.64 molecular solution of KCl have been added.

TABLE 15.

Composition of the solution. s. w. = sea-water.		Relative concentration of potassium cation, its concentration in natural sea-water being 1.	Relative rate of 3 normal medusæ.[1]	Relative rate of a normal medusa.[2]	Relative rate of a ring of subumbrella tissue.
Natural sea-water		1.00	[3]100	[4]100	[5]100
100 c.c. s. w. + 0.25 c.c. KCl		1.16	100		
100	0.5	1.28	103		
100	0.75	1.44	122		
100	1.	1.6	112		
100	1.25	1.76	69		
100	1.5	1.92	54		
100	1.75	2.07	67		
100	2.	2.22	61.5		
100	2.25	2.37	75		
100	2.5	2.53	81	33	101
100	2.75	2.69	67		
100	3.	2.84	67		
100	3.25	2.98	37		
100	3.5	3.13	22		
100	3.75	2.27			
100	5.	4.			85
100	7.5	5.3			62

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[1] These medusæ recovered their normal rates after being returned to sea-water.
[2] Stopped in less than one hour. This medusa recovered its normal pulsation when returned to sea-water.
[3] See curve CD, fig. 12.
[4] See curve AB, fig. 12, and record, fig. 13.
[5] See curve EF, fig. 12.

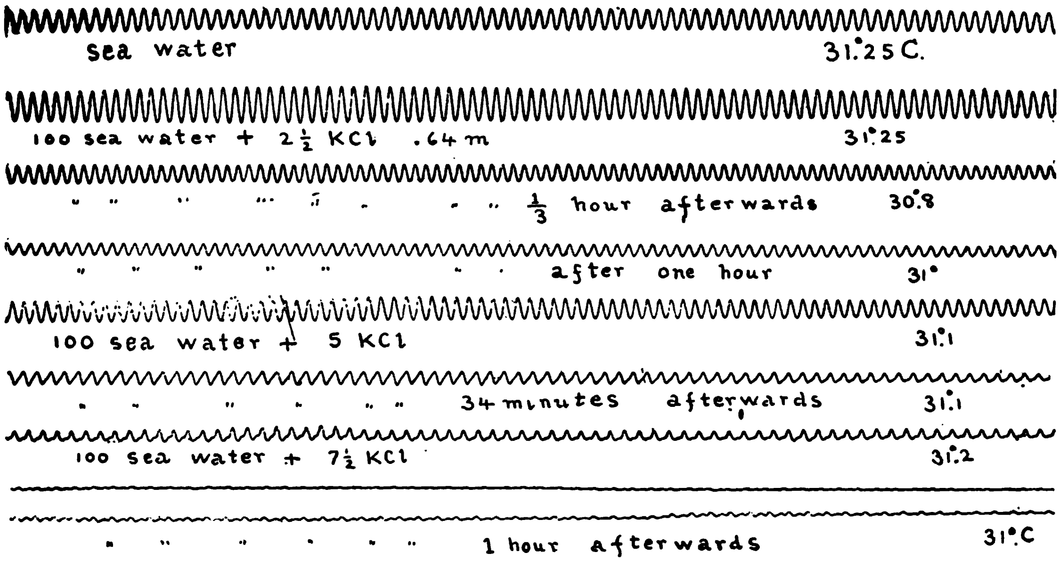

FIG. 13.

If we place normal medusæ in a solution having the proportions of 100 c.c. sea-water + 1 c.c. of 0.64 molecular KCl, their rates are increased and maintained thus for more than 24 hours. This solution gives the potassium ion a concentration 1.6 times as great as in natural sea-water. Any further increase in the concentration of the potassium ion causes an initial excitement, but this is soon followed by a loss in rate. Rings are not so sensitive and the nerves may maintain a normal rate in a solution having 2.5 times the concentration of the potassium ion in sea-water, but a still further concentration causes a decline in rate after temporary, initial excitement.

It has long been known that weak concentrations of potassium cause initial excitement followed by loss of rate. It is, however, interesting to see that a very weak concentration may be a permanent stimulant, for it shows that potassium acts in a manner similar to that of sodium, its close associate in Group I of the periodic system. Like potassium, sodium itself produces sustained increase in rate in weak excess, but depression in stronger concentrations. Also in stronger concentrations its initial effect is to stimulate, but depression soon follows. Sodium also is slightly toxic, potassium even more so. Thus in all these respects potassium resembles sodium, but its metallic properties are more decided, as indeed we would expect from its higher atomic weight.

EFFECTS OF CALCIUM.

It is difficult to determine the effects of the calcium ion *per se*, for (as is well known) it associates itself or combines with the sodium ion, possibly forming a sodium-potassium ion proteid, and it enables the sodium to counteract the negative effect of magnesium.

If *Cassiopea* be placed in a van't Hoff's solution resembling sea-water, but lacking calcium, all movement soon ceases, but pulsation begins to be revived when we add a concentration of calcium equal to 0.4 or 0.5 that found in natural sea-water. Pulsation is most rapid, however, when the concentration of the calcium ion is about 2.5 times that found in natural sea-water. Further additions of calcium cause increasing depression and movements usually cease when the solution contains 7 times the concentration of calcium found in sea-water. Such a solution is not toxic, however, for complete recovery takes place almost immediately if the medusæ be restored to sea-water.

It is interesting to see that half the normal concentration of calcium suffices to restore pulsation, for half the normal concentration of sodium will accomplish the same end, provided magnesium, calcium, and potassium be present in normal concentration.

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III.

THE LAW GOVERNING THE LOSS OF WEIGHT IN STARVING CASSIOPEA.

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One plate and twenty-one figures.

THE LAW GOVERNING THE LOSS OF WEIGHT IN STARVING CASSIOPEA.

By Alfred Goldsborough Mayer.

METHODS.

Before being weighed each medusa was dried upon filter paper so as to remove the water adherent to its surface. It was then weighed in 25 c.c. of sea-water on a delicate balance, the weight of the medusa being determined by subtracting the weight of the water and the glass beaker serving as its container.

The medusæ were starved in sea-water which had been passed through two glass funnels, each containing a double layer of the best quality of Chardin's filter paper, thus removing the zoöplankton upon which *Cassiopea* feeds. In some of the experiments the sea-water after having been passed through Chardin's filters was refiltered through porcelain or it was rendered sterile in so far as animal life was concerned by heating it to 71° C. and then restoring the evaporation by distilled water. These additional precautions were, however, found to be unnecessary for the Chardin filters removed all or practically all food from the water.

The medusæ were always starved in the purest sea-water which was either dipped from the ocean in glass or canvas buckets or pumped into glass reservoir tanks through hard-rubber pipes by means of a hard-rubber pump. In running comparative series care was taken that the sea-water in which each medusa was starved had had the same history; *i. e.*, was gathered in the same way, stored in the same tank, etc., for every medusa in the series. Whenever possible the medusæ were starved side by side in one and the same glass aquarium, but when this was impossible the aquaria were of similar size and form and were placed side by side, so as to be subjected to similar environmental changes.

EXPERIMENTS.

It is a pleasure to acknowledge my indebtedness to Dr. Francis Gano Benedict for important advice and highly appreciated kindness, and to Mr. Joseph C. Bock, of the Nutrition Laboratory of the Carnegie Institution of Washington, for having determined the nitrogen content of the medusæ.

In common with other scyphomedusæ, *Cassiopea xamachana* consists of a large central mass of gelatinous substance, which is covered with a thin layer of cellular tissue, the weight of the gelatinous substance being very great in proportion to that of the cellular elements of the medusa's body.

When the animal starves, this gelatinous substance decreases in volume and apparently serves as the chief store of food for the starving animal.

If W be the weight of the medusa when starving begins, aW may represent the decline in weight due to loss of body-substance and of water at the end of the first day, so that at the end of the first day the weight of the medusa is $W - aW = W(1 - a)$.

Similarly, at the end of the second day the weight is $W(1 - a) - aW(1 - a) = W(1 - a)^2$.

Hence the weight y after starving x days is $y = W(1 - a)^x$ where a may be called the index of catabolism, the rate of starvation increasing as a increases.

This formula accords fairly well with the facts of observation, as will be apparent from an inspection of tables 4 to 27 and the curves accompanying them. At times the observed weights are above and at other times below the theoretical curve, but the general average in table 1 for 30 normal medusæ starved in the diffuse daylight of the laboratory is practically coincident with the curve $y = W(1 - 0.567)^x$ for a period of 19 days starving in filtered sea-water.

Table 1 and figure 1 show the average decline in weight deduced from six series of experiments upon 30 medusæ of *Cassiopea xamachana* starved 19 days *in diffuse daylight* in doubly filtered sea-water. The weights are reduced to a scale of 100 grams at the beginning of the experiment: Thus, if a medusa weighed 10 grams at the beginning and 5 grams at the end of a certain number of days, these weights would appear as 100 and as 50, respectively, in the accompanying table:

TABLE 1.

Days elapsed.	Observed average weight.	Calculated weight.[1]
0	100	100
2	88.5	88.97
4	77.82	79.16
6	71.35	70.43
9	59.73	59.11
12	50.44	49.61
15	42.08	41.64
19	32.9	32.9

[1] Calculated according to the formula $y = 100(1 - 0.0567)^x$.

This simple law governing the loss of weight in *Cassiopea* leads to the conjecture that the chemical constitution of the animal does not change, but that one and the same class of substances serves to maintain the medusa throughout the period of its starvation. It is well known that in vertebrates a very different condition pertains, for the starving animal at first mainly consumes its store of glycogen, oxidizing it into carbohydrate; then the fats are chiefly drawn upon, so that a starving vertebrate contains relatively less fat and more proteid and water than a normal creature. Thus there is a progressive change in the chemical constitution of the body of the animal.

This appears not to be the case in *Cassiopea*, and this supposition receives further support from an analysis of the percentage of nitrogen in the body, which appears to range from 2.121 to 2.997 per cent of the dried weight of the animal, the variation being independent of the number of days the animal has starved. Hence it seems probable that starving neither increases nor decreases the percentage of nitrogen. The results are shown in table 2, wherein it appears that there is no coördination between the number of days the medusa has been starved and the percentage of nitrogen it contains. The medusæ were desiccated at 100° C. at Tortugas, Florida, and the dried substance was then sent to Boston for analysis.

TABLE 2.

Days starved.	Live weight in grams just before being killed.	Dried weight in grams.	Per cent of dried to living weight.	Per cent of nitrogen to dried weight.
0	109.055	5.401	4.9	2.566
0	109.641	5.43	4.9	2.147
3	91.9	4.425	4.8	2.476
4	157.71	6.91	4.3	2.48
6	....[1]	5.972	....	2.997
10	94.380	4.38	4.6	2.121
12	86.72	4.362	5.0	2.226
14	40.288	1.93	4.8	2.451
16	83.232	3.981	4.8	2.543
			Av. 4.762	Av. 2.445

[1] Not weighed.

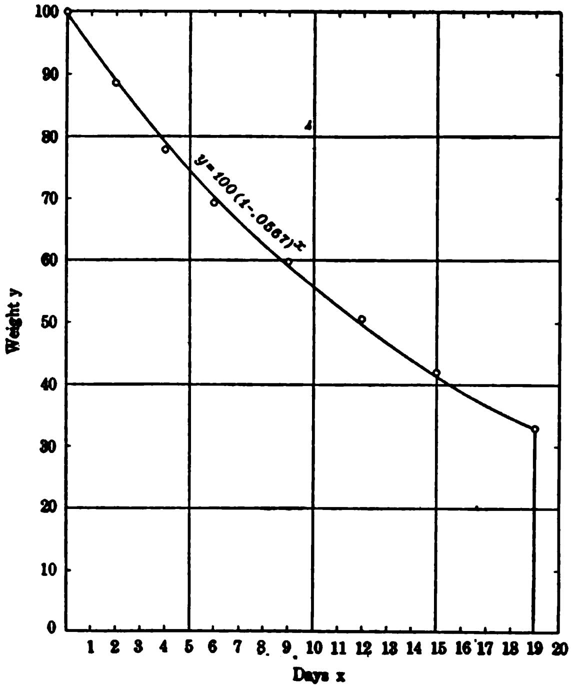

FIG. 1.

It appears also that the dried weight is about 4.76 per cent[1] of the living weight, and that this ratio does not change as the animal starves. Thus the living weight bears a practically constant ratio to the weight of the solid substance of the animal, and the law governing the loss of rate may be determined either from the one or the other, the living weight being preferable on account of its greater magnitude and the consequent lessening of the degree of error in its determination. Hence the reason for the validity of the simple law that governs the loss of weight in the starving jellyfish is that no appreciable chemical change occurs in the composition of its body-substances, and there is no appreciable selective use of different substances at different times during the progress of starvation.

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[1] Of this 4.7 per cent, 3.5 per cent consists of the salts of sea-water; and thus the actual body-substance of the medusa is only 1.2 per cent, the animal containing 98.8 per cent of water.

Sections show that as a physical result of starvation, the cells become reduced in size, many degenerate and disappear, and the cell boundaries tend to become indistinct; moreover, the gelatinous substance becomes vacuolated and the muscular tonus is largely lost, so that the bell-rim bends upward and inward in a balloon-like manner, as is shown in plate 1, figure A, which represents 6 medusæ that have been starved 41 days and whose combined weights have declined from 74.95 to 2.78 gm., all being still alive. In fact, throughout these series of experiments no medusa has been lost through natural death, although some of the series of observations upon medusæ starved in the dark were terminated on account of the development of local maceration due possibly to ineffectually checked bacterial activity.

Plate 1, figure B, gives an enlarged view of the smallest of these medusæ shown in plate 1, figure A, and one may observe its crumpled, shrunken bell, the loss through coalescence of the mouths, and the absorption of the mouth-arm appendages. Indeed, the first organs to disappear are usually the mouths and their appendages, and thus after being starved for about 3 weeks recovery may become impossible through the inability of the animal to capture the zoöplankton upon which the medusa feeds. Experiments indicate that they do not feed upon diatoms, but in common with the corals, as determined by Vaughan, their food appears to be exclusively of an animal nature, the smaller forms of the zoöplankton being taken into the numerous mouths on the mouth-arms.

It is known that much of the nannoplankton can pass through the best filters, but one was unable to discover any specimens of the zoöplankton in the doubly filtered sea-water used in these experiments. Nevertheless, medusæ with their stomachs removed starve more rapidly than do animals with their stomachs intact but regenerating their bell-rims. The medusæ which possess stomachs are, however, not called upon to regenerate any of their parts and are thus subjected to less drain upon their resources.

The value of a in the formula $y = W(1 - a)^x$ gives us a fair measure of the rate of loss of weight. It may be called the "coefficient of negative metabolism," for as a increases the loss of weight increases in like ratio.

In the experiments recorded in this paper a ranges as shown in table 3.

It appears from table 3 that *Cassiopea* thrives best and starves at its slowest rate if maintained in large quantities of sea-water changed only once in each 24 hours, thus resembling the conditions of the semistagnant lagoons in which the medusa lives.

TABLE 3.

Medium in which medusæ were starved and conditions of the experiment.	Value of a when medusæ are starved in diffuse daylight of laboratory.	Value of a when medusæ are starved in dark.	Tables referred to.
Normal medusæ each in 6 liters or more of doubly filtered sea-water changed once in every 24 hours.	0.046 to 0.075; av. 0.0567	0.0639 to 0.075; av. 0.0695	4 to 10 and 14
Medusæ regenerating bell-rim but with stomach and mouth-arms intact, each in 6 liters or more of doubly filtered water changed once in each 24 hours.	0.0681 to 0.0689; av. 0.0685	0.0511 to 0.0538; av. 0.052	19 to 25
Regenerating disks with stomach and mouth-arms removed, each in 6 liters or more of doubly filtered water changed once in each 24 hours.	0.06 to 0.1034; av. 0.0815	0.074 to 0.0937; av. 0.0892	18 to 25
Normal medusæ in 400 c.c. of doubly filtered water changed once in each 24 hours.	0.1145	0.1	11 and 12
Normal medusæ in large quantities of doubly filtered constantly changing sea-water.	0.1015 to 0.15; av. 0.125	0.165 to 0.185; av. 0.175	15 and 16
Normal medusa in 6 liters of sea-water sterilized by heating it to 71° C., and then cooling it to normal temperatures. The water being changed once in each 24 hours.	0.055	....	17

The relative rates of starvation both in darkness and in diffuse daylight, shown by medusæ under other conditions, are as follows, the results being derived from table 3.

Normal medusæ in large amounts of stagnant water	1.0
Medusæ regenerating their bell rims, but with stomach and mouth-arms intact	0.96
Medusæ starving without stomachs	1.27
Normal medusæ in small aquaria of 400 c.c. capacity	1.7
Normal medusæ in constantly running water	2.4
Normal medusæ in water sterilized by heating it to 71° C.	1.0

Although for the first week or more the regenerating medusæ generally lose weight more rapidly than do the normal animals the final rate of starvation appears to be practically the same in normal and in regenerating medusæ provided their stomachs be present, and this is in accord with the well-known converse fact that the rate of regeneration is independent of the food-supply, starving animals regenerating quite as rapidly as well-fed ones.

In this connection it appears from an inspection of the curves accompanying tables 4 to 27, that if the starving medusæ lose but little weight for a day or two, a succeeding period of abnormally rapid loss is apt to ensue and thus the animals tend to follow the theoretical curve $y = W(1 - a)^x$.

Cassiopea lives in shallow lagoons of semistagnant water, where it lies fully exposed to the hot sun and, as table 3 shows, it loses weight slowly when kept in quantities of water of about 6 liters per animal in aquaria the water of which is changed only once in each 24 hours. If the water be changed constantly, however, or the medusæ be kept in running water, they starve more rapidly than if maintained in stagnant water.

It will be recalled that Allen and Nelson[1] found that if sea-water be heated to 70° C. practically all life within it is killed, and the water remains sterile unless reinfected.

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[1] Allen, E. J., and E. W. Nelson (1910). Quarterly Journal of Microscopical Science, London, vol. 55, pp. 361–431; also, Journal of Marine Biology Association, vol. 8.

Bearing this in mind, the sea-water was doubly filtered and then heated to 71° C. after which it was allowed to cool in sterilized glass vessels protected from dust. Medusæ kept in this sterilized water, whether in the dark or in daylight, lost weight no more rapidly than would have been the case had they been starved in sea-water that had been merely passed through four Chardin filter papers without being sterilized by heating.

When sea-water is heated to 71° C., however, some of its dissolved air is expelled, and in order fully to compensate for this medusæ were starved in a control sea-water solution which had been placed in a vacuum under the receiver of an air pump, thus expelling far more air than was lost by the sea-water which had been heated to 71° C. It was found, however, that the commensal plant-cells of the medusa render the animal comparatively independent of the oxygen of the sea-water, provided an excess of CO_2 be absent, and thus the medusa starves at practically the same rate in heat-sterilized, air-exhausted, or normal filtered sea-water. In fact, it starves at this same rate in sea-water which has been doubly filtered through the Chardin filters and then refiltered through porcelain to extract bacteria and nannoplankton. Putter's[1] "dissolved food" of sea-water may, therefore, still be available as food even after all organisms have been taken out of the sea-water, and it appears that such organisms are not necessary to initiate a process of assimilation.

If the stomach be intact, the animals starve less rapidly than if it be removed. Animals whose stomachs have been cut off can regenerate new gastro-vascular centers, but this is rarely done, and it so happened that none of the disks studied by me succeeded in replacing their stomachs, although all made more or less progress in regeneration.

In order to determine the effect of the regeneration, we compared the starving rate of these agastric medusæ with others having stomachs intact but regenerating their bell-rims, an annulus of the bell being cut off close to the outer edge of the stomach, as is shown in plate 1, figure C.

Table 3 and tables 18 to 25 show that disks without stomachs lose weight about 1.25 times as rapidly as do regenerating medusæ with stomachs intact.

Carbon dioxide plays a conspicuous rôle in controlling the activities of the medusa, for Harvey[2] found that during the day the velocity of nerve-conduction is greater than during the night, and this fact is possibly due to the variation in the supply of oxygen conditioned by symbiotic algæ in the subumbrella tissue of the medusa.

It was found that if we make a weak solution of rosolic acid in sea-water and divide it into two equal volumes in two separate containers and then cut a medusa into halves and place each half in a volume of the rosolic-acid solution, it will be observed that if one half of the medusa be kept in diffuse daylight and the other half in darkness, the rosolic acid surrounding the darkened half of the medusa loses color and fades rapidly, while the fluid surrounding the half of the medusa which is in daylight fades more slowly. This and the lime-water test in magnesium-free sea-water show that the medusa gives off CO_2, and that in diffuse daylight less CO_2 is given off than in darkness; the difference being apparently due to photosynthesis on the part of the commensal plant-cells or zoöxanthellæ which infest the gelatinous substance immediately under the external cell-layers of the medusa.

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[1] The most complete presentation of Putter's views is given in his recent work "Die Ernährung der Wasserthiere und der Stoffansalt der gewasser" (Jena, 1910).

[2] Harvey, E. Newton. Carnegie Institution of Washington, Year Book No. 10, 1911, p. 131.

Tables 3, 11, and 12 show that when the medusæ are confined in a small volume of water they starve more rapidly than if kept in large aquaria. They also pulsate slower when confined in small aquaria, and a slight excess of CO_2 brings them to rest, but even when not moving they lose weight far more rapidly than if pulsating in normal sea-water, the toxic effect of CO_2 being very noticeable. If a few bubbles of CO_2 be passed through sea-water the medusæ cease to pulsate; plate 1, figure A, is derived from a photograph of animals treated in this manner. Upon replacing the medusæ in normal sea-water pulsation is immediately renewed, no permanent toxic effects appearing until they have been subjected to the CO_2 for about 24 hours.

The influence of CO_2 is probably counteracted by the well-known photosynthesis due to the commensal plant-cells or zoöxanthellæ.

When the medusæ are starved in diffuse daylight these plant-cells become more and more crowded as the medusa declines in bulk, and after several days of starving they begin to escape early in the morning, when the swarming zoöxanthellæ penetrate the ectoderm and pass out into the ocean, where they swim actively, and finally settle upon those lighted parts of the cylindrical glass aquarium which are away from the source of light, the swarming cells being entrapped in the lighted meniscus of the aquarium in accordance with the Jennings reaction. When, on the other hand, the medusæ are starved in the dark, the algal cells are rendered inactive and do not escape into the water. Medusæ starved in the light become dark brown in color after the first 10 days, for the plant-cells remain alive, although apparently unhealthy and probably inactive.

When the medusæ are starved in darkness many of the plant-cells die and dissolve *in situ* in the gelatinous substance. Not all of these cells die, however, for a few still remain alive and apparently healthy after being more than a month in the dark, and if the medusa be replaced in the light these cells propagate and again provide the animal with a normal supply of zoöxanthellæ. Sections show that when the medusæ starve in the dark the gelatinous substance becomes vacuolated and edemic, due possibly to the unreduced excess of CO_2 in the tissues. Thus the medusæ when starved in the dark appear to suffer from lack of oxygen, being in effect partially suffocated. Yet even when the plant-cells have been greatly reduced through 26 days of starvation in the dark, the medusæ can still pulsate for 2 or more hours at nearly a normal rate in sea-water that has been boiled to expel its oxygen. It at one time seemed that these plant-cells played a significant rôle in maintaining the weight of the medusa when starved in daylight, but further experiments have shown that this is not the case, for the medusæ sometimes starve at a more rapid rate in diffuse daylight than in the dark, although the reverse is usually the case.

It is not the object of this paper to present an exhaustive review of all previous researches upon the metabolism of inanition in invertebrates, because previous studies do not touch upon the chief subject of this study—the law of the decline of weight. A brief mention of certain important sources of general information should, however, be presented.

The earliest work appears to be that of Dumas and Milne-Edwards (1820, Annales Chim. et Physique, tome 14; and also in the Comptes Rendus, Paris, 1843, tome 17, p. 531) upon the metabolism of bees.

Peligot (1865, Comptes Rendus, tome 61, p. 866; and 1867, Annales Chim. et Physique, tome 12, p. 445) studied silkworms in the same connection.

Especially worthy of mention is the work of Slowtzoff[1] (1904), who found that when starved the vineyard slug consumed about 25.74 per cent of its total weight and about 28.41 per cent of its store of energy. The relation between the organic and inorganic substance remains the same in the normal and in the starved animals. The loss of vital substances consists of fat, carbohydrates, and water in the proportions of carbohydrate 93.98 per cent, fat 78.51 per cent, and water about 30.02 per cent, while the loss of albumen was computed to be 23.70 per cent. As a result of starvation the soft parts of the animal acquire an increase of insoluble salts to about 35.9 per cent. During the period of starvation the slugs consume 4.84 calories of energy per kilogram of their weight per 24 hours. The phosphoric albumens were largely retained, only about 19 per cent of these being parted with.

In 1901 the same author[2] starved the dung beetle, *Geotrypes stercorarius* and found that in from 5 to 11 days it lost about 21 per cent of its original weight, the loss appearing to be chiefly water and fat.

Schulz[3] starved *Planaria lactea* and states that after 6 months without food some of the worms were reduced to about one-tenth their original size. There were, however, considerable individual differences, some of the animals being three times as large as the smallest after all had starved for 6 months. Schulz made a histological study of the starved worms and found that some of the cells were killed as a result of the starvation, while others had degenerated or become reduced in size. The epithelium tends to fuse and its cells to degenerate into a syncytium, very much as we have observed in *Cassiopea*.

Perkins[4] observed that the cells of the attached hydroid larvæ of *Gonionemus murbachii* degenerated in aquaria, where presumably they were unfed or poorly nourished, and that the larvæ became amœboid and changed into creeping plasmodia without cell boundaries. Thacher[1] also observed a process of degeneration in hydroid colonies of *Eudendrium*, *Pennaria*, and *Campanularia* kept in aquaria. The polypites of these forms are absorbed, the degenerating cells of the entoderm and finally the ectoderm of the hydranths being turned into the digestive tract of the stem.

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[1] Slowtzoff, B. (1904). Beitrage zur vergleichenden Physiologie der Hungerstoffwechsels, Mittheil. 2, Der Hungerstoffwechsel der Weimbergschnecke (Hofmeister's Beitrage).

[2] Slowtzoff, B. (1909). *Idem.*, Mittheil 5, Der Hungerstoffwechsel der Mistkäfer (*Geotrypes stercorarius*). Biochemischer Zeitschrift, Bd. 19, p. 504.

[3] Schulz, E. (1904). Ueber Reductionen, I, Ueber Hungerscheinungen bei *Planaria lactea*. Archiv für Entwicklungsmechanik der Organismen, Bd. 18, pp. 555–577, Taf. 34, 11 figm.

[4] Perkins, H. F. (1902). Proc. Acad. Natural Sci., Philadelphia, vol. 54, p. 765; also: 1902. Biol. Bulletin Woods Hole, vol. 3, pp. 172–180.

By contrast with the few studies upon invertebrates many researches have been conducted upon the metabolism of matter and energy in the body of starving vertebrates. To properly review this literature would carry us too far afield and indeed a text-book would be required for the purpose, but the literature of the subject, in so far as it relates to man and the higher vertebrates, has been exhaustively reviewed by Atwater and Langworthy (1897) in their "Digest of Metabolism Experiments" (434 pp., Bulletin No. 45, United States Department of Agriculture, Office of Experiment Stations). One should also consult such later works upon vertebrates as Atwater and Benedict (1903), Benedict (1907), Weber (1902), Ergebnisse der Physiologie; Schaefer (1898), Text Book of Physiology, vol. 1, p. 891; Manca (1902), Pembrey and Spriggs (1904), Abderhalden, Bergell, and Dorpinghaus (1904), Hatai (1904), Cathcart and Fawsitt (1907), and Maignon (1908), and others referred to by these authors, the list of papers cited by Pembrey and Spriggs in the Journal of Physiology (Cambridge, vol. 31, pp. 320–345), and by Benedict (1907), being especially enlightening.

EXPLANATION OF THE TABLES.

In the figures accompanying tables 4 to 25, when the medusæ are starved in darkness their observed weights are indicated by black dots; when starved in the diffuse light of the laboratory their weights are shown by circles.

Tables 4 to 25 refer to *Cassiopea xamachana* and table 26 to the allied species *C. frondosa*, which follows a law in its starvation similar to that of *C. xamachana*.

Table 4 shows the decline in weight of 6 normal medusæ of *Cassiopea xamachana* starved 41 days in filtered sea-water, in the *diffuse light* of the laboratory, at temperatures ranging from 27.3° to 30.1° C., from June 18 to July 29, 1911, at Tortugas, Florida. The medusæ were kept in a 6-liter glass aquarium, the water being changed once in every 24 hours. Compare this with table 10 to see the effect of darkness upon medusæ subjected to conditions as nearly as possible identical with the medusæ starved in daylight. The curve $y = 75(1 - 0.075)^x$ does not accord well with the facts, but the curve represented by $y = 75(1 - 0.058)^x$ accords fairly well with the observed decline in weight up to the sixteenth day, after which it departs more and more widely from the observed weights and is unsatisfactory. (See table 5.)

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[1] Thacher, H. F. (1903). Biol. Bulletin Woods Hole, vol. 4, pp. 96–98.

TABLE 4.

Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	74.95	75
1	73.36	....
2	68.315	64.13
3	65.345	....
4	62.975	54.9
5	59.065	....
6	55.37	46.95
7	51.62	....
8	47.955	40.2
9	45.16	....
10	42.69	34.35
11	40.225	....
12	36.28	29.4
13	33.825	....
14	31.185	25.2
15	29.11	....
16	28.065	21.52
17	27.77	....
18	24.78	18.45
19	23.15	....
20	21.44	15.75
21	18.95	....
22	18.18	13.50
23	17.01	....
24	15.67	11.55
25	14.46	....
26	12.98	9.90
27	11.73	....
28	10.37	8.47
29	9.21	....
30	8.03	7.23
31	7.26	....
32	6.63	6.18
33	6.04	....
34	5.34	5.29
35	4.84	....
36	4.46	4.53
37	4.05	....
38	3.67	3.87
39	3.39	....
40	3.07	3.31
41	2.78	3.07

[1] According to formula $y = 75(1 - 0.075)^x$.

TABLE 5.

Days elapsed.	Observed weights.	Calculated weight.[1]
0	74.95	75
5	59.065	55.65
10	42.69	41.7
15	29.11	30.6
20	21.44	22.72
25	14.46	16.8
30	8.03	12.45
35	4.84	9.22
40	3.07	6.87

[1] According to formula $y = 75(1 - 0.058)^x$.

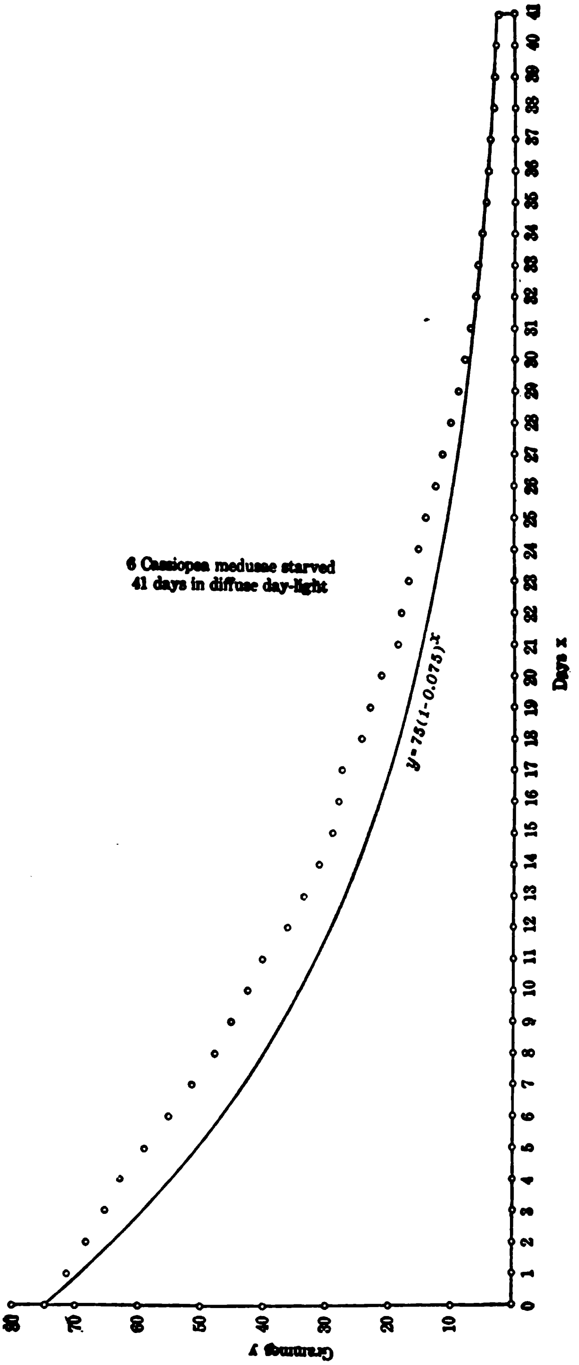

Diagram illustrating Table 4.

Table 6 shows the decline in weight of 10 normal medusæ of *Cassiopea xamachana* starved 25 days in filtered sea-water in the *diffuse light* of the laboratory from June 22 to July 17, 1910, at Tortugas, Florida. The medusæ were kept in a 6-liter glass aquarium, the water being changed once in each 24 hours.

TABLE 6.

Days elapsed.	Observed weight, in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	130.75	131	9	92.14	85.67	18	56.79	56.07
1	128.96	124.97	10	88.71	81.74	19	53.45	53.58
2	119.58	119.21	11	86.24	78.08	20	51.81	51.09
3	115.34	113.71	12	79.54	74.41	21	48.20	48.73
4	108.97	108.6	13	72.31	71	22	45.84	46.5
5	106.58	103.49	14	69.27	67.73	23	44.71	44.41
6	103.55	98.77	15	66.81	64.58	24	42.78	42.31
7	97.60	94.19	16	....[2]	61.7	25	39.7	40.35
8	97.22	89.87	17	61.24	58.82	....	....	....

[1] According to formula $y = 131(1 - 0.046)^x$.

[2] No weighing.

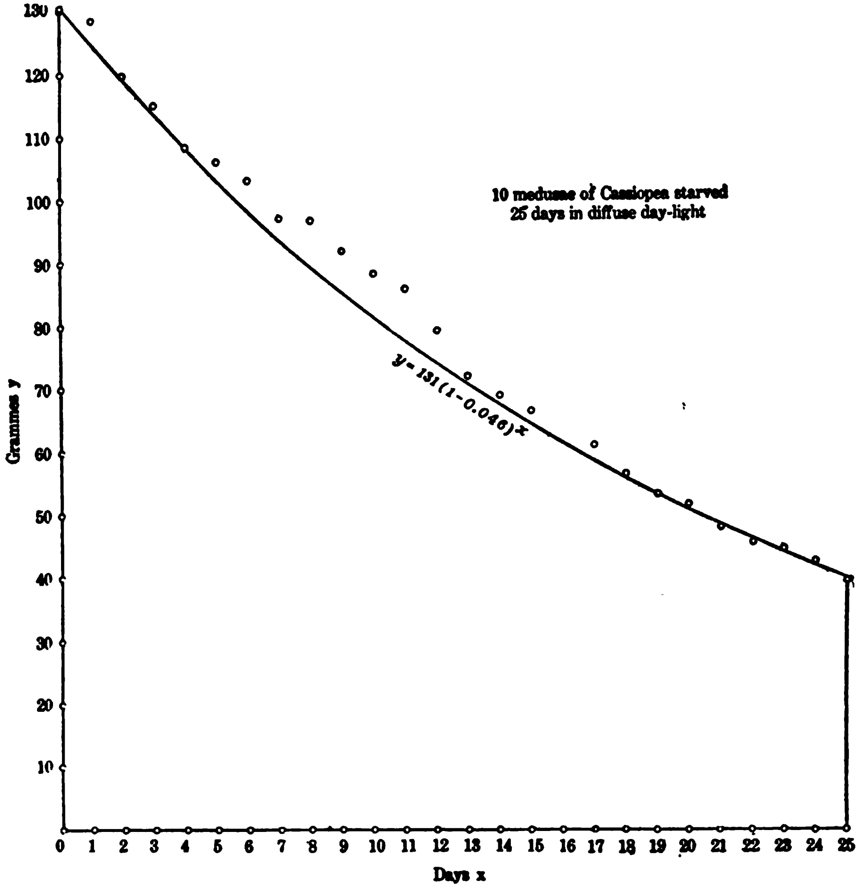

Diagram illustrating Table 6.

Table 7 shows the decline in weight of 11 normal medusæ of *Cassiopea xamachana* starved 22 days in filtered sea-water in the *diffuse light* of the laboratory, at temperatures ranging from 27.1° to 30.1° C., from June 19 to July 11, 1911, at Tortugas, Florida. The medusæ were kept in a 6-liter glass aquarium, the water being changed once in each 24 hours.

TABLE 7.

Days elapsed.	Observed weight in grams.	Calculated. weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	496.9	497	8	328	321.56	16	223.2	208.24
1	457	470.66	9	315	304.66	17	206.9	197.31
2	432.1	445.8	10	306	288.86	18	191.2	186.87
3	399.69	421.95	11	290.99	273.35	19	179.2	176.93
4	385.1	399.59	12	278.95	258.94	20	172.2	167.49
5	369.3	377.22	13	266.3	245.02	21	161.63	158.54
6	365.5	358.83	14	249.5	232.10	22	150.09	150.25
7	345	339.45	15	240.8	219.67	....	....	....

[1] According to formula $y = 497(1 - 0.0529)^x$.

REMARKS.—Free-swimming algal cells were given off from these medusæ every morning soon after daylight for the first week. These plant-cells collected on the side of the cylindrical glass aquarium which was away from the light.

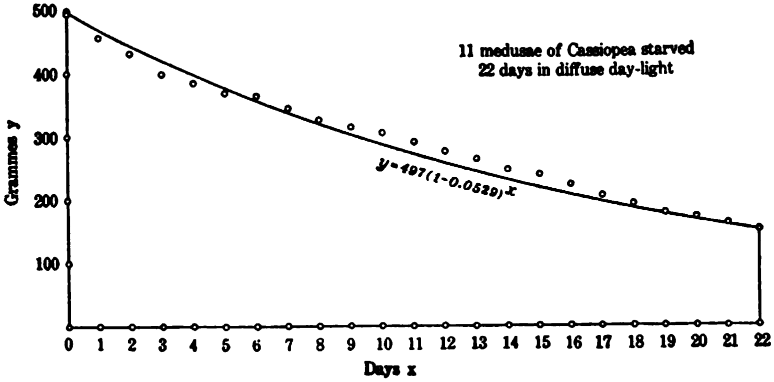

Diagram illustrating Table 7.

Table 8 shows the decline in weight of a single normal medusa of *Cassiopea xamachana* starved 17 days in the *diffuse daylight* of the laboratory in filtered sea-water at temperatures ranging from 26.5° to 30.1° C., from June 22 to July 9, 1911, at Tortugas, Florida. The medusa was kept in a 6-liter glass aquarium, the water being changed once in each 24 hours.

TABLE 8.

Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	261.95	262	10	133.1	132.31
1	248.5	244.7	11	128	123.66
2	243.9	288.46	12	121.95	115.28
3	231.5	213.53	13	112	108.21
4	215.8	199.38	14	110.2	102.61
5	192.75	186.28	15	101.8	94.06
6	185.7	173.97	16	93.2	87.77
7	166.7	162.44	17	81.9	82
8	158.3	151.7			
9	144.7	141.74			

[1] According to the formula $y = 262(1 - 0.66)^x$.

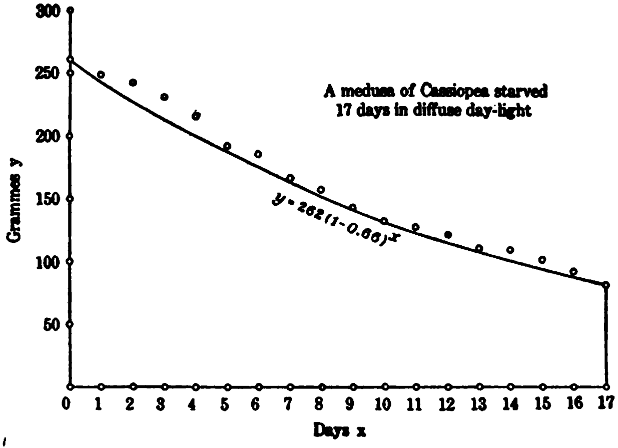

Diagram illustrating Table 8.

Table 9 shows the decline in weight of two normal medusæ of *Cassiopea xamachana* starved 25 days in *diffuse daylight*, in filtered sea-water, at 27.5° to 30° C., from June 28 to July 23, 1912, at Tortugas, Florida. The medusæ were placed in a glass aquarium holding 6 liters, the water being changed once in each 24 hours.

TABLE 9.

Days elapsed.	Medusa *A*.		Medusa *B*.	
	Observed weight in grams.	Weight.[1]	Observed weight in grams.	Weight.[2]
0	42.62	42.62	54.9	54.9
4	31.73	32.00	36.48	43.2
7	26.94	25.76	34.94	36.18
10	20.52	20.80	29.08	30.19
13	16.48	16.79	25.51	25.25
17	13.25	12.57	19.41	19.87
21	10.19	9.46	14.99	15.70
25	7	7	12.35	12.35

[1] Calculated from formula $y = 42.62(1 - 0.0692)^x$.
[2] Calculated from the formula $y = 54.9(1 - 0.0579)^x$.

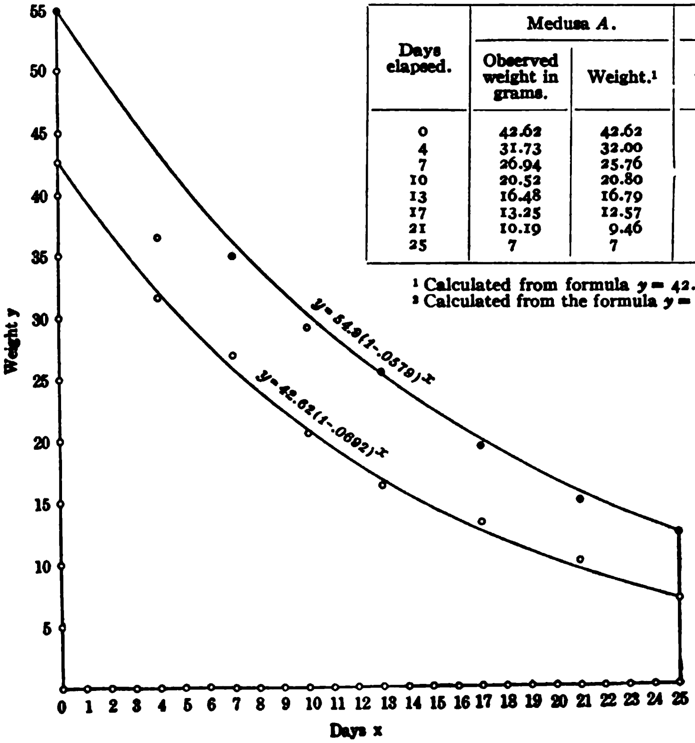

Diagram illustrating Table 9.

Table 10 shows the decline in weight of 6 normal medusæ of *Cassiopea xamachana* starved 26 days in filtered sea-water in the *dark* at temperatures ranging from 27.3° to 30.1° C., from June 18 to July 14, 1911, at Tortugas, Florida.[1] The medusæ were placed in a 6-liter glass aquarium, the water being changed once in each 24 hours. The temperature conditions were similar to those maintained for the medusæ whose rates of decline are shown in table 4.

TABLE 10.

Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	85.59	85.6	9	41.03	42.47	18	23.24	20.97
1	79.57	79.18	10	40.96	39.20	19	20.965	19.43
2	75	73.27	11	39.4	36.29	20	18.85	17.98
3	70.9	67.71	12	33.535	33.56	21	17.56	16.69
4	63.35	62.66	13	28.51	31.07	22	15.31	15.41
5	56.2	57.87	14	27.23	28.76	23	14.38	14.21
6	52.6	53.59	15	27.12	26.54	24	13.07	13.18
7	51.705	49.56	16	26.49	24.57	25	10.78	12.16
8	42.09	45.88	17	24.87	22.77	26	8.99	11.30

[1] According to the formula $y = 85.6(1 - 0.075)^x$.

REMARKS.—These 6 medusæ were captured in the moat of Fort Jefferson, Tortugas, on June 16 and weighed for the first time on June 18, 1911. The smallest was about 25 mm. wide on June 18. On June 22 the smallest was only 8.5 mm. wide, and by June 25 and 26 the medusæ had begun to macerate.

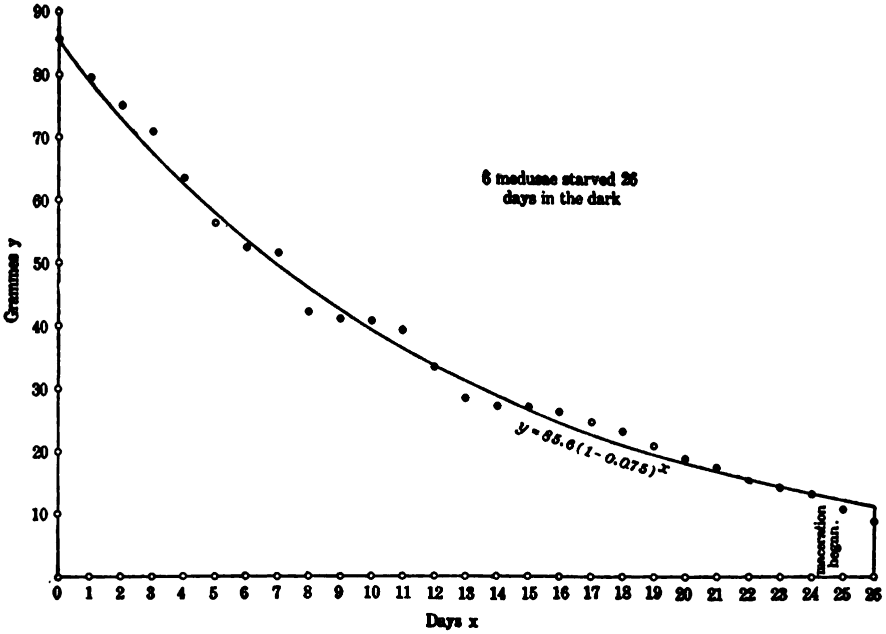

Diagram illustrating Table 10.

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[1] For the first 14 days the medusæ remained in complete darkness, not being exposed for an instant to the light. On the fourteenth day they were observed in diffuse light, and it appeared that they were all blue-translucent, not greenish gray, as are the normal medusæ. The algal cells were then greatly reduced in number. The medusæ were then replaced in the dark as soon as possible, and remained in darkness until the end of the experiment, excepting that from the fourteenth to the twenty-sixth day they were weighed in the diffuse light of the laboratory but were replaced in the dark room immediately after each weighing.

Table 11 shows the decline in weight of a single medusa of *Cassiopea xamachana* starved 19 days in filtered sea-water, in the ***dark***, at temperatures ranging between 27.3° and 30.1° C., from June 30 to July 19, 1911, at Tortugas, Florida. During the first 10 days this medusa was placed in 400 c.c. of sea-water which was changed once each day. After this it was placed in a glass vessel holding about 6 liters and the water was changed constantly by mechanical means, thus giving an abundant supply of fresh sea-water. Compare this with table 12 which shows the starving rate of a medusa maintained in daylight.

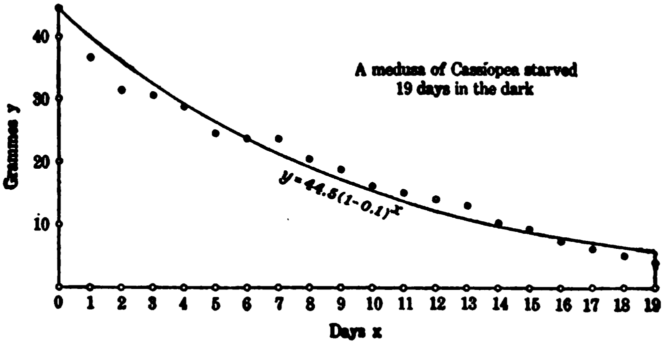


Diagram illustrating Table 11.

TABLE 11.

Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	44.51	44.5	7	23.66	21.27	14	10.45	10.19
1	36.69	40.05	8	20.49	19.13	15	9.45	9.17
2	31.44	36.24	9	18.99	17.24	16	7.70	8.25
3	30.59	32.44	10	16.05	15.53	17	6.30	7.39
4	28.825	29.19	11	15.03	13.97	18	5.23	6.68
5	24.485	26.25	12	14.01	12.57	19	4.23	6
6	23.77	23.63	13	13.12	11.303	....	....	....

[1] According to the formula $y = 44.5(1 - 0.1)^x$.

REMARKS.—On July 19 maceration began and the experiment was discontinued.

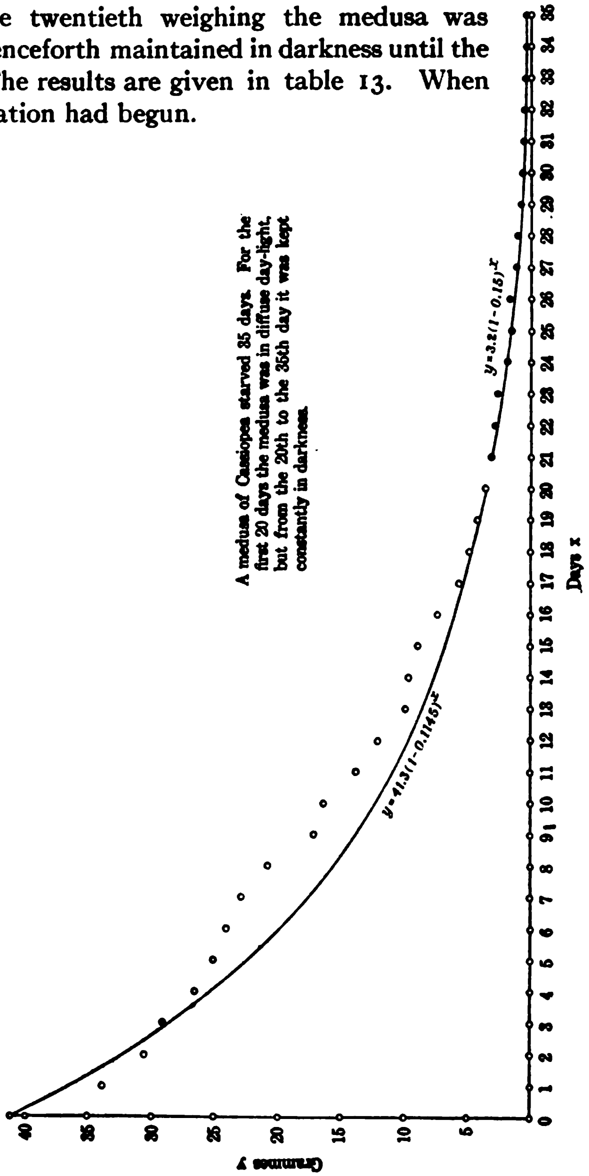
Table 12 shows the decline in weight of a single medusa of *Cassiopea xamachana* starved 20 days in the *diffuse daylight* of the laboratory, and then for 15 more days in the dark, in filtered sea-water at temperatures ranging between 27.3° and 30.1° C., from June 30 to August 4, 1911, at Tortugas, Florida. During the first 10 days this medusa was placed in 400 c.c. of sea-water which was changed once each day. After this it was placed in a glass vessel holding about 6 liters and the water was changed constantly by mechanical means, thus giving an abundant supply of sea-water. The conditions of the experiment are comparable with those shown on table 11, excepting that table 12 applies to a medusa starved in daylight, while table 11 shows the decline in weight of one starved in darkness.

Immediately after the twentieth weighing the medusa was placed in the dark and thenceforth maintained in darkness until the end of the experiment. The results are given in table 13. When the records ceased, maceration had begun.

TABLE 12.

Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	41.32	41.3
1	33.92	36.55
2	30.52	32.38
3	29.18	28.66
4	26.66	25.4
5	25.09	22.47
6	24.03	19.91
7	22.93	17.64
8	20.77	15.61
9	17.63	13.84
10	16.41	12.22
11	13.80	10.82
12	12.02	9.58
13	9.86	8.51
14	9.66	7.52
15	8.93	6.65
16	7.34	5.91
17	5.64	5.20
18	4.875	4.63
19	4.27	4.09
20	3.66	3.63

[1] According to formula $y = 41.3(1 - 0.1145)^x$.



A medusa of Cassiopea starved 35 days. For the first 20 days the medusa was in diffuse day-light, but from the 20th to the 35th day it was kept constantly in darkness.

Diagram illustrating Tables 12 and 13.

TABLE 13.

Days elapsed in darkness.		Observed weight.	Calculated weight.[1]	Days elapsed in darkness.		Observed weight.	Calculated weight.[1]	Days elapsed in darkness.		Observed weight.	Calculated weight.[1]
21	1	3.2	3.2	26	6	1.54[2]	1.42	31	11	0.68	0.64
22	2	2.83	2.72	27	7	1.205	1.206	32	12	0.54	0.54
23	3	2.53	2.31	28	8	1.01	1.02	33	13	0.54	0.45
24	4	1.98	1.96	29	9	0.87	0.84	34	14	0.45	0.39
25	5	1.52	1.64	30	10	0.75	0.74	35	15	0.29	0.34

[1] According to the formula $y = 3.2(1 - 0.15)^x$.

[2] An error in weighing.

Table 14 shows the decline in weight of a normal medusa of *Cassiopea xamachana* starved 25 days in the ***dark***, in filtered sea-water, at 27.5° to 30° C., from June 28 to July 23, 1912, at Tortugas, Florida. The medusa was placed in a glass aquarium holding 6 liters, the water being changed once in each 24 hours.

TABLE 14.

Days elapsed.	Observed weight of medusa in grams.	Calculated. weight.[1]	Days elapsed.	Observed weight of medusa in grams.	Calculated weight.[1]
0	32.77	32.77	13	15.09	13.93
4	21.58	25.17	17	12.52	10.65
7	19.54	20.61	21	9.04	8.12
10	17.34	16.91	25	6.28	6.28

[1] According to formula $y = 32.77(1 - 0.0639)^x$.

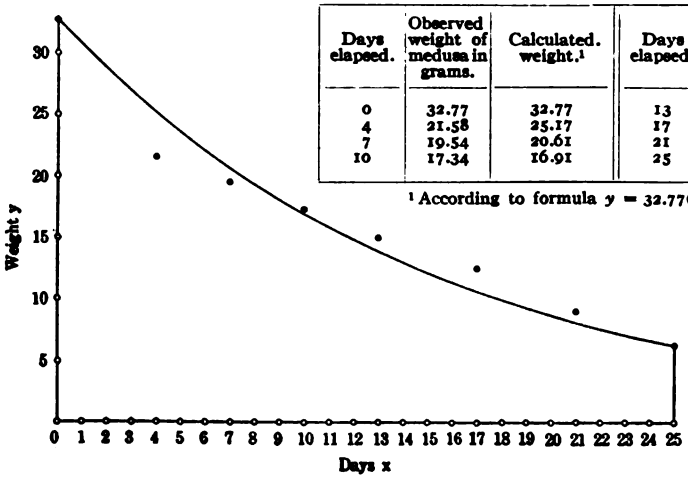


Diagram illustrating Table 14.

Table 15 shows the decline in weight of two medusæ of *Cassiopea xamachana* starved in ***diffuse daylight*** in constantly changing, filtered sea-water at temperatures ranging from 27.3° to 30.1° C., at Tortugas, Florida, from July 10 to August 4, 1911. Compare this with table 16.

These medusæ were starved side by side in the same 6-liter glass vessel and were subjected to the same changes of temperature, water, light, etc. Being in running water, they lost weight more rapidly than one would expect had they been in semi-stagnant water.

TABLE 15.

Days elapsed.	Medusa *A*.		Medusa *B*.		Days elapsed.	Medusa *A*.		Medusa *B*.	
	Observed weight.	Expected weight.[1]	Observed weight.	Expected weight.[2]		Observed weight.	Expected weight.[1]	Observed weight.	Expected weight.[2]
0	40.58	40.6	37.16	37.1	13	10.26	10.11	8.20	9.18
1	40.26	36.48	33.5	33.37	14	9.34	9.05	7.67	8.18
2	37.08	32.76	26.88	29.95	15	8.56	8.16	7.38	7.39
3	31.6	29.43	23.53	26.90	16	8.09	7.31	7.00	6.65
4	27.03	26.47	20.03	24.15	17	7.21	6.58	6.515	5.95
5	23.475	23.79	18.35	21.70	18	6.09	5.93	5.39	5.35
6	19.79	21.36	16.53	19.47	19	5.41	5.32	4.73	4.79
7	18.15	19.20	13.78	17.50	20	4.73	4.75	3.99	4.31
8	17.41	17.25	13.48	15.72	21	4.17	4.26	....	....
9	14.17	15.47	12.43	14.12	22	3.99	3.85	....	....
10	12.79	13.93	11.19	12.67	23	3.48	3.46	....	....
11	11.92	12.50	10.30	11.37	24	3.08	3.11	....	....
12	10.96	11.25	8.80	10.22	25	2.52	2.79	....	....

[1] Calculated from formula $y = 40.6(1 - 0.1015)^x$.
[2] Calculated from formula $y = 37.16(1 - 0.102)^x$.

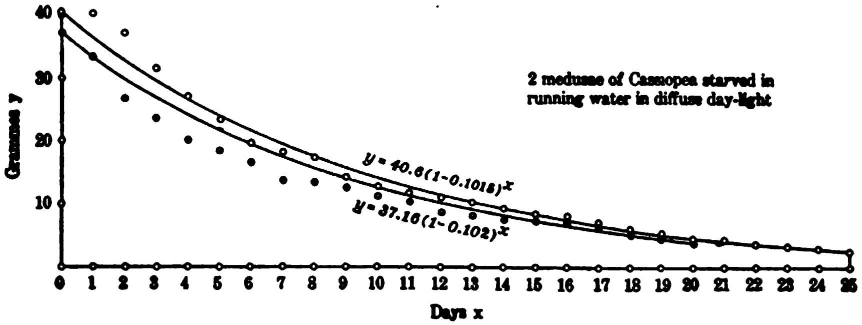


Diagram illustrating Table 15.

Table 16 shows the decline in weight of two medusæ of *Cassiopea xamachana* starved 9 days in the *dark* in constantly changing, filtered sea-water at temperatures ranging from 27.3° to 30.1° C., at Tortugas, Florida, from July 10 to 19, 1911. Compare this with table 15.

TABLE 16.

Days elapsed.	Medusa *C*.		Medusa *D*.	
	Observed weight.	Expected weight.[1]	Observed weight.	Expected weight.[2]
0	51	....	36.37	36.37
1	[3]51.78	51	34.17	29.64
2	44.09	42.58	24.88	24.15
3	38.86	35.55	20.83	19.68
4	30.5	29.68	16.88	16.04
5	28.28	24.79	15.33	13.06
6	24.13	20.71	14.19	10.66
7	19.25	17.29	9.62	8.69
8	17.61	14.43	7.49	7.06
9	11.97	12.03	5.79	5.78

[1] According to formula $y = 51(1 - 0.165)^x$.
[2] According to formula $y = 36.37(1 - 0.185)^x$.
[3] Medusa *C* did not begin to lose weight until after it had been confined 24 hours in the dark. The commensal plant-cells seem to be of no aid to the medusa when in pure, constantly changing sea-water. The medusæ live, in nature, only in semi-stagnant salt lagoons.

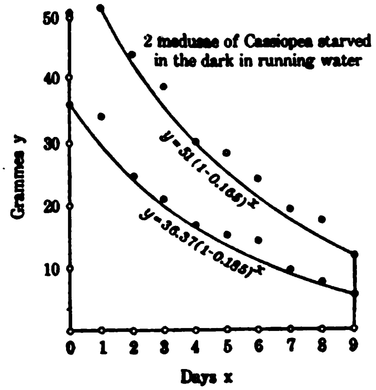


Diagram illustrating Table 16.

TABLE 17.

Days elapsed.	Medusa *A*.		Medusa *B*.		Medusa *C*.	
	Weights in water which had been tested to 70° C.	Calculated weights.[1]	Weights in water which had been partially exhausted of air.	Calculated weights.[2]	Weights in normal filtered sea-water.	Calculated weights.[3]
0	49.567	50	40.45	....	35.83	35.8
2	46.61	44.62	40.69	40.5	29.94	31.82
4	45.13	39.9	[4]38.57	36.08	28.44	28.32
6	38.39	35.6	32.18	32.19	24.61	25.16
8	33.09	31.8	31.64	28.7	22.94	22.37
10	29.7	28.3	29.28	25.55	20.93	19.95
12	26.19	25.25	25.73	22.76	19.18	17.61
14	23.7	22.7	21.82	20.29	16.77	15.65
16	19.83	20.2	18.28	18.1	13.93	13.99
18	18.18	17.8	16.51	16.51	12.45	12.45
20	15.7	15.4	14.36	14.75	11.1	11.06

[1] According to the formula $y = 50 (1 - 0.0553)^x$.
[2] According to the formula $y = 40.5(1 - 0.0559)^x$.
[3] According to the formula $y = 35.8 (1 - 0.057)^x$.
[4] Probably an error.

Table 17 shows the decline in weight of three normal specimens of *Cassiopea xamachana* starved for 20 days at Tortugas, Florida, in filtered sea-water. One medusa (*A*) was maintained in water which had been heated to 71° C. and then cooled to normal temperatures. Another (*B*) was kept in sea-water from which most of the dissolved air had been exhausted by means of an air pump. The third medusa (*C*) was kept in normal filtered sea-water.

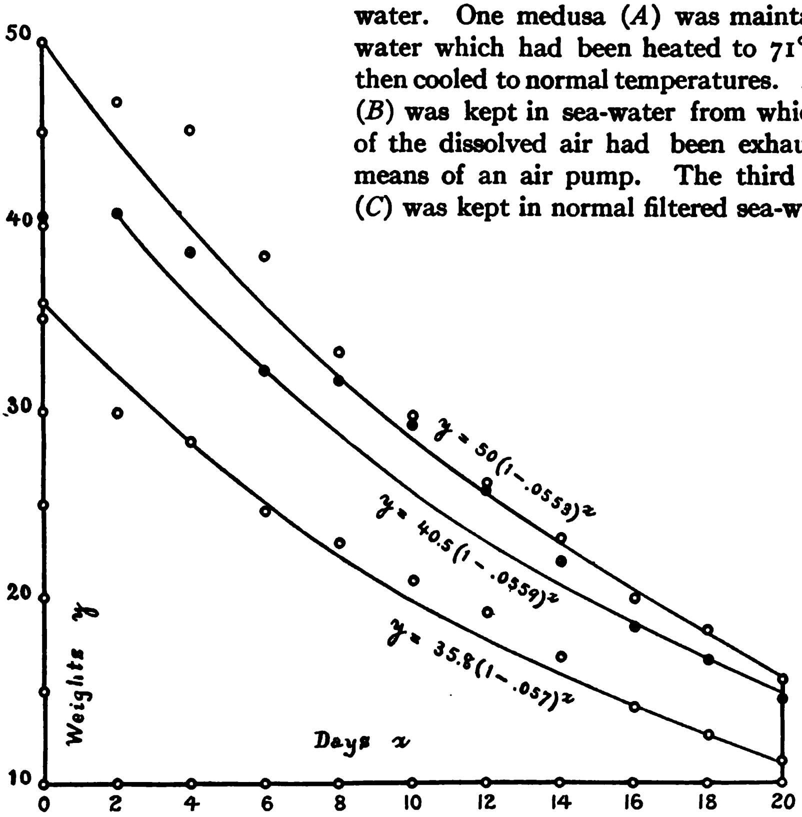


Diagram illustrating Table 17.

Table 18 shows the decline in weight of three half disks of *Cassiopea xamachana* each starved in 6 liters of water in diffuse daylight, together with

TABLE 18.

Days elapsed.	Disk *A*.		Disk *B*.		Disk *C*.	
	Weights of half-disk starved in sea-water which had been heated to 71° C.	Weights calculated.[1]	Weights of half-disk starved in sea-water which had been partially exhausted of air.	Weight calculated.[2]	Half of disk starved in normal filtered sea-water.	Weight calculated.[3]
0	23.05	23.05	20.035	20.03	18.535	18.5
2	19.46	20.33	17.07	17.68	15.68	16.27
4	17.815	17.96	14.72	15.62	13.83	14.27
6	15.55	15.89	12.33	13.82	11.84	12.52
8	13.82	14	11.38	12.2	11.04	11
10	12.16	12.4	10.17	10.8	9.04	9.65
12	10.63	11.1	9.31	9.66	7.7	8.57
14	9.68	9.69	8.39	8.42	7.21	7.43
16	8.79	8.55	7.63	7.44	6.4	6.52
18	7.61	7.56	6.84	6.58	5.65	5.72
20	6.71	6.71	6.2	5.82	5.03	5.03

[1] According to formula $y = 23(1 - 0.06)^x$.
[2] According to formula $y = 20(1 - 0.06)^x$.
[3] According to formula $y = 18.5(1 - 0.063)^x$.

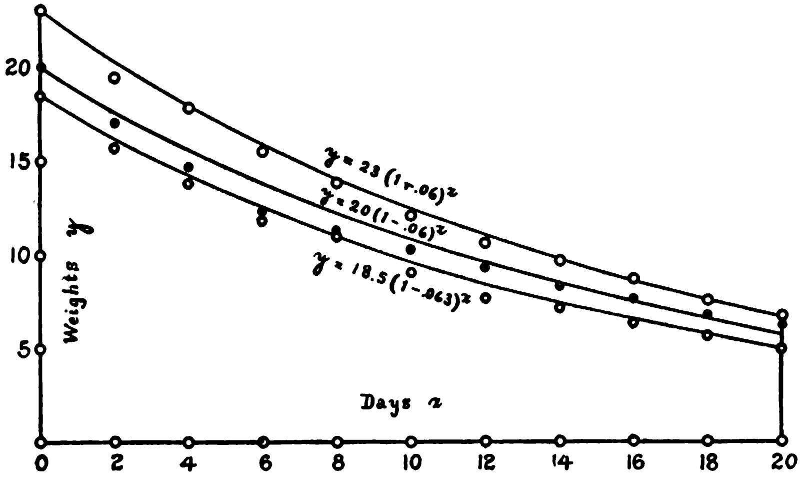


Diagram illustrating Table 18.

the normal medusæ described on table 17. The stomach and mouth-arms were cut off and the disk cut into halves. One half (Disk *A*) was starved in doubly filtered sea-water which had been heated to 71° C.; the other half of the same disk (Disk *B*) was starved in doubly filtered sea-water which had been placed in a vacuum to expel most of its dissolved air. The third half disk (Disk *C*) was taken from another medusa and was starved in normal doubly filtered sea-water.

Table 19 shows the decline in weight of two regenerating specimens of *Cassiopea xamachana* starved 19 days in diffuse daylight, in filtered sea-water

TABLE 19.

Days elapsed.	Observed weight of regenerating disk in grams.	Weight of disk.[1]	Weight of regenerating medusa in grams.	Weight of regenerating normal medusa.[2]
0	25.48	25.48	41.87	41.87
2	20.15	20.48	33.98	36.2
6	9.66	13.24	24.30	27.3
9	8	9.55	18.97	22.02
12	6.52	6.88	14.23	17.79
15	5.06	4.97	13.87	14.36
19	4.21	3.21	10.81	10.81

[1] Calculated according to formula $y = 25.48(1 - 0.1034)^x$.
[2] Calculated according to formula $y = 41.87(1 - 0.0689)^x$.

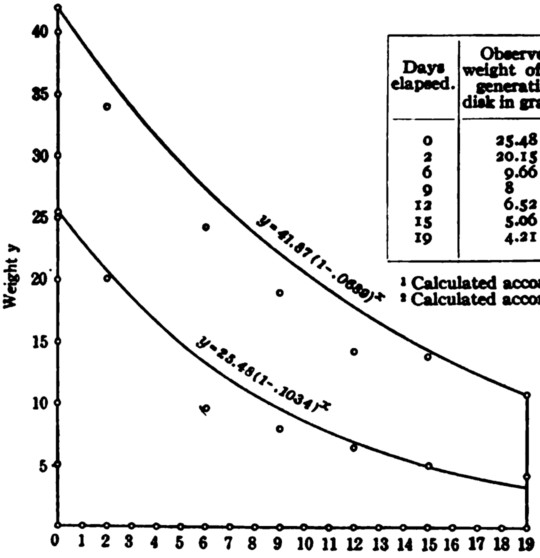


Diagram illustrating Table 19.

at 27.5° to 30° C., from June 26 to July 15, 1912, at Tortugas, Florida. One of these specimens was a disk from which the stomach and mouth-arms had been cut off, and the other was a medusa with stomach and mouth-arms intact but with its bell-rim cut off. The two were maintained in one and the same 6-liter glass aquarium jar throughout the experiment, and were thus subjected to identical environmental conditions. The water was changed once in each 24 hours.

Table 20 shows the decline in weight of two regenerating species of *Cassiopea xamachana* starved 19 days in ***diffuse daylight,*** in filtered sea-water at 27.5° to 30° C., from June 26 to July 15, 1912, at Tortugas, Florida. One of these specimens was a disk from which the stomach and mouth-arms had been removed, and the other specimen was a medusa with stomach and mouth-arms intact but with the bell-rim removed. The two were maintained in one and the same 6-liter glass aquarium throughout the experiment, and were thus subjected to identical environmental conditions. The water was changed once in every 24 hours.

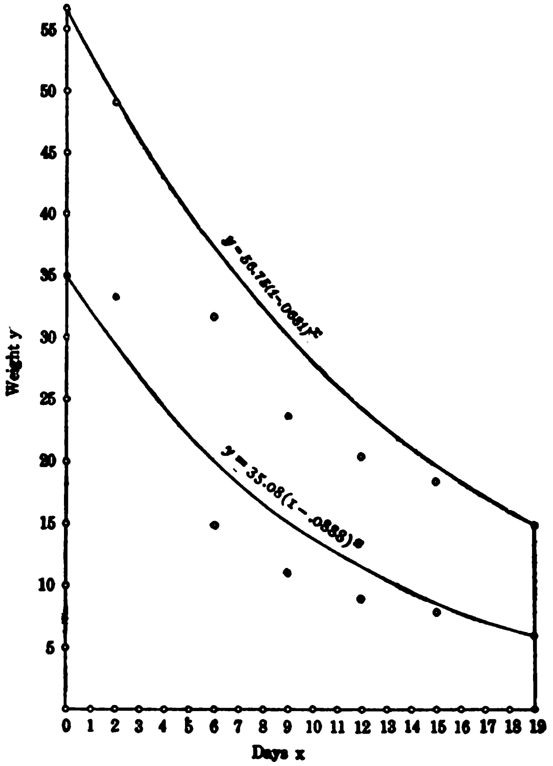


Diagram illustrating Table 20.

TABLE 20.

Days elapsed.	Observed weight of regenerating disk in grams.	Weight of disk.[1]	Observed weight of regenerating medusa in grams.	Weight of regenerating medusa.[2]
0	35.08	35.08	56.75	56.75
2	33.28	29.11	49.11	49.26
6	14.86	20.06	31.76	37.17
9	11.04	15.19	23.63	30.07
12	8.98	11.51	20.43	24.34
15	7.9	8.7	18.45	19.69
19	6	6	14.88	14.88

[1] Calculated according to formula $y = 35.08(1 - 0.0888)^x$.
[2] Calculated according to formula $y = 56.75(1 - 0.0681)^x$.

Table 21 shows the decline in weight of the two halves of a disk of *Cassiopea xamachana* starved in the diffuse daylight of the laboratory at Montego Bay, Jamaica, from February 27 to March 18, 1912, in water ranging from 24.5° to 28° C. The stomach and mouth-arms of the medusa were removed and the disk cut into two nearly equal halves, both of which were maintained in one and the same glass aquarium holding about 6 liters. The water was changed once in every 24 hours.

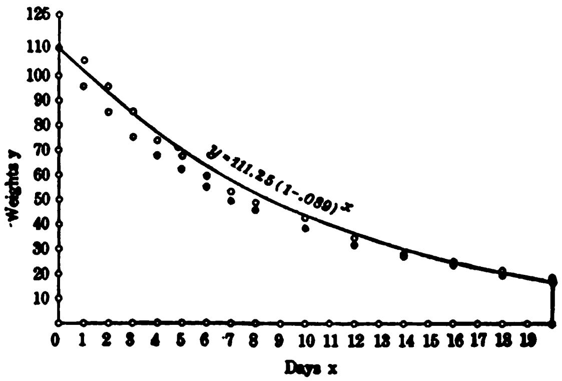


Diagram illustrating Table 21.

TABLE 21.

Days elapsed.	Medusa *A*.		Medusa *B*.	
	Weight.	Calculated weight.[1]	Weight.	Calculated weight.[2]
0	111.25	111.25	125.93	125.93
1	95.61	101.35	106.13	114.33
2	85.41	92.34	95.61	103.76
3	75.43	84.11	85.33	94.32
4	67.96	76.66	73.96	85.62
5	62.39	69.77	67.51	77.69
6	55.29	63.63	59.81	70.52
7	49.53	57.96	53.06	64.09
8	45.76	52.73	48.86	58.18
10	38.49	43.83	42.72	47.97
12	32.84	36.38	34.56	39.53
14	27.31	30.15	28.65	32.61
16	23.88	25.03	25.08	26.81
18	19.76	20.8	21.61	22.16
20	17.12	17.24	18.26	18.27

[1] According to the formula $y = 111.25(1 - 0.089)^x$.
[2] According to the formula $y = 125.93(1 - 0.092)^x$.

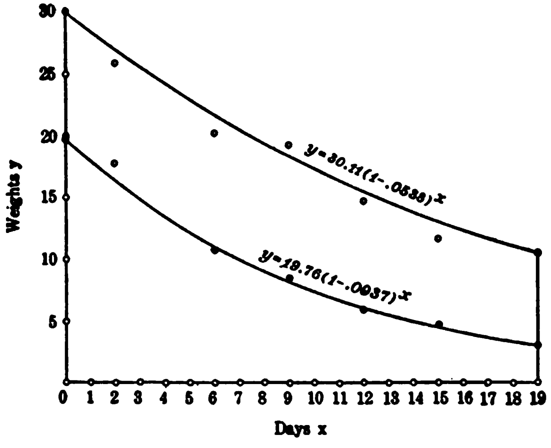


Diagram illustrating Table 22.

Table 22 shows decline in weight of two regenerating specimens of *Cassiopea xamachana* starved 19 days in the *dark*, in filtered sea-water, at temperatures ranging from 27.5° to 30°C., from June 26 to July 15, 1912, at Tortugas, Florida. One was a disk deprived of its stomach and mouth-arms, and the other was a medusa regenerating its bell-rim, but with stomach and mouth-arms intact.

TABLE 22.

Days elapsed.	Observed weight of regenerating disk in grams.	Weight of disk.[1]	Observed weight of regenerating medusa in grams.	Weight of regenerating medusa.[2]
0	19.76	19.76	30.11	30.11
2	17.8	16.22	25.89	26.95
6	10.8	10.95	20.21	21.59
9	8.54	8.14	19.22	18.31
12	5.94	6.07	14.76	15.5
15	4.79	4.62	11.7	13.13
19	3.05	3.05	10.53	10.53

[1] Calculated according to formula $y = 19.76(1 - 0.0937)^x$.
[2] Calculated according to formula $y = 30.11(1 - 0.0538)^x$.

The two specimens were maintained in one and the same 6-liter aquarium jar throughout the experiment, and were thus subjected to identical environmental conditions. The water was changed once in every 24 hours.

Table 23 shows the decline in weight of two regenerating specimens of *Cassiopea xamachana*, one being a disk without stomach or mouth-arms, and the other a medusa with stomach and mouth-arms intact but with the rim of the bell cut off. Thus each medusa was regenerating, the disk being without and the medusa with its stomach. These two specimens were kept side by side in one and the same 6-liter glass aquarium, in *constant darkness*, in filtered sea-water at 27.5° to 30° C. for 19 days from June 26 to July 15, 1912. The water was changed once in every 24 hours.

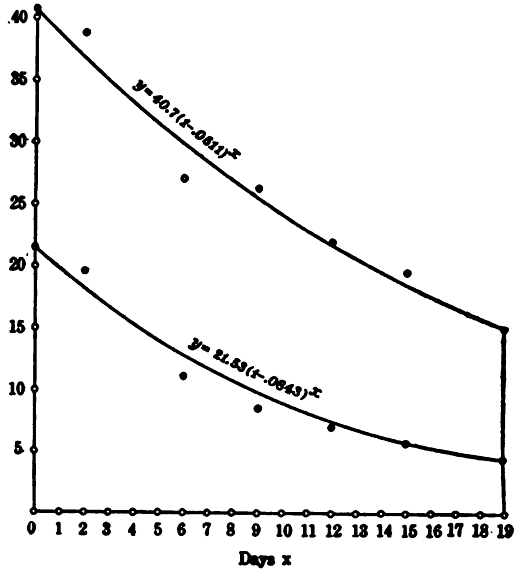


Diagram illustrating Table 23.

TABLE 23.

Days elapsed.	Observed weight of regenerating disk in grams.	Weight of regenerating disk.[1]	Observed weight of regenerating medusa in grams.	Weight of regenerating medusa.[2]
0	21.53	21.53	40.7	40.7
2	19.59	18.02	38.8	36.65
6	11.12	12.83	27.12	29.72
9	8.56	9.74	26.33	25.4
12	7.07	7.46	21.96	21.71
15	5.76	5.74	19.48	18.55
19	4.04	4.04	15.05	15.05

[1] Calculated according to formula $y = 21.53(1 - 0.0843)^x$.
[2] Calculated according to formula $y = 40.7(1 - 0.0511)^x$.

Table 24 shows the decline in weight of two regenerating specimens of *Cassiopea xamachana* starved 19 days in the *dark*, in filtered sea-water, at temperatures ranging from 27.5° to 30° C., from June 26 to July 15, 1912, at Tortugas, Florida. One of the specimens was a disk deprived of both stomach and mouth-arms, and the other was a medusa with the rim of its bell cut off but with stomach and mouth-arms intact; thus both were regenerating new tissue. They were kept side by side in one and the same 6-liter aquarium jar, and were thus subjected to similar conditions. The water was changed once in every 24 hours.

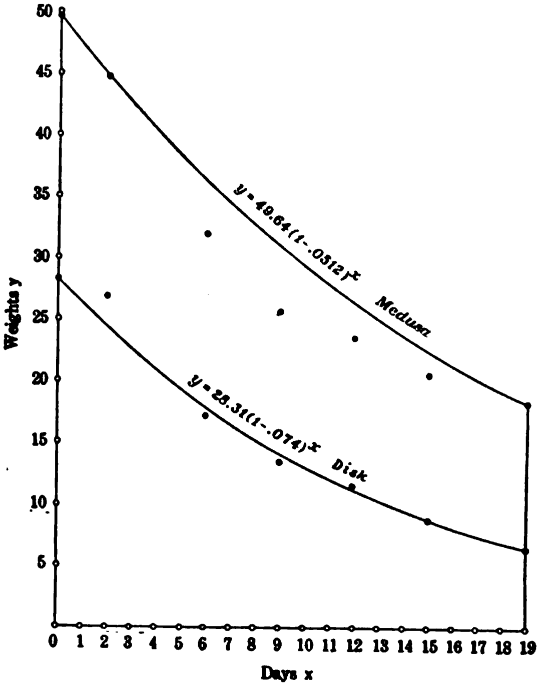


Diagram illustrating Table 24.

TABLE 24.

Days elapsed.	Observed weight of regenerating disk in grams.	Weight of disk.[1]	Observed weight of regenerating medusa in grams.	Weight of regenerating medusa.[2]
0	28.31	28.31	49.64	49.64
2	26.91	24.25	44.61	44.65
6	17.23	17.83	31.97	36.19
9	13.57	14.16	25.71	30.88
12	11.64	11.24	23.61	26.36
15	8.8	8.91	20.62	22.51
19	6.58	6.58	18.24	18.24

[1] Calculated according to formula $y = 28.31\ (1 - 0.074)^x$.
[2] Calculated according to formula $y = 49.64(1 - 0.0512)^x$.

Table 25 shows the decline in weight of the two halves of a disk of *Cassiopea xamachana* without stomach or mouth-arms. One of these halves, *A*, was starved in the diffuse daylight of the laboratory and the other, *B*, in constant darkness, for 18 days, from February 28 to March 18, 1912, at Montego Bay, Jamaica, at temperatures ranging from 24.5° to 28° C. The two halves were maintained side by side in one and the same 6-liter glass aquarium and the water was changed once in each 24 hours.

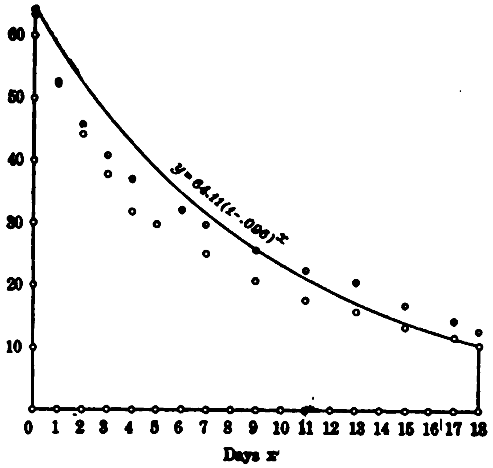


Diagram illustrating Table 25.

TABLE 25.

Days elapsed.	Medusa *A*.		Medusa *B*.	
	Weight, starved in diffuse daylight.	Weight.[1]	Weight, starved in darkness.	Weight.[2]
0	64.11	64.11	63.46	63.46
1	52.18	57.96	52.53	58.16
2	44.28	52.38	45.91	53.12
3	37.88	47.38	40.93	48.61
4	31.98	42.83	37.06	44.48
5	29.9	38.72	....	....
6	....	....	32.27	37.25
7	25.07	31.61	29.91	34.08
9	20.9	25.83	25.69	28.56
11	17.69	21.09	22.49	23.35
13	15.83	17.24	20.6	20.
15	13.45	14.11	16.73	16.75
17	11.86	11.54	15.46	14.02
18	10.40	10.45	12.83	12.83

[1] According to formula $y = 64.11(1 - 0.096)^x$.
[2] According to formula $y = 63.46(1 - 0.085)^x$.

TABLE 26.

Days elapsed.	Half-disk starved in daylight.	Decline in weight.[1]	Half-disk starved in dark.	Decline in weight.[2]
0	73.01	73	66.11	66.11
1	63.13	65.12	58.28	58.83
2	53.06	58.11	51.33	52.35
3	41.49	51.83	43.09	46.60
4	33.91	46.21	37.11	41.44
5	31.01	41.25	....	36.88
6	....	....	30	32.85
7	26.51	32.78	25.66	29.22
8	....	....	....	....
9	22.44	26.06	19.12	23.14
10	....	....	....	....
11	18.56	20.8	16.87	18.31
12	....	....	....	....
13	16.22	16.50	14.83	14.54
14	....	....	....	....
15	13.47	13.14	12.06	11.5
16	....	....	....	....
17	11.17	10.44	10.54	9.12
18	9.40	9.34	8.06	8.06

[1] Calculated in accordance with formula $y = 73(1 - 0.108)^x$.
[2] Calculated according to formula $y = 66.11(1 - 0.11)^x$.

Table 26 shows the decline in weight of two halves of a disk of *Cassiopea frondosa* deprived of its stomach. One of these halves was starved in diffuse daylight and the other in the dark from February 28 to March 18, 1912, at Montego Bay, Jamaica.

TABLE 27.

Days elapsed.	Medusa *A*.		Medusa *B*.	
	Observed weights.	Weights.[1]	Observed weights.	Weights.[2]
0	60.96	61	57.54	57.5
2	51.17	50.08	49.3	49.45
4	42.26	42.27	45.36	42.26
6	34.01	35.19	39.06	36.51
8	29.33	29.33	31.41	31.41
10	23.59	24.77	27.6	27.25
12	21.2	20.37	24.72	23.23

[1] Calculated from the formula $y = 61(1-0.1673)^x$.
[2] Calculated from the formula $y = 57.5(1-0.14)^x$.

Table 27 shows the decline in weight of two normal medusæ of *Cassiopea xamachana* starved each in one liter of sea-water, changed once in each 24 hours, and kept in the diffuse daylight of the laboratory at Tortugas, Florida, from June 8 to 20, 1913. One medusa, *A*, was starved in sea-water which had been passed through two glass funnels each holding two sheets of Chardin filter paper. The other medusa, *B*, was starved in sea-water, which, in addition to having been filtered through the aforesaid Chardin filters, was also filtered through a bacteria-proof porcelain filter. It appears that all food had been removed from the water by the Chardin filters and the medusa in the bacteria-free sea-water starved somewhat more slowly than the one in the sea-water which had not been passed through the porcelain filter.

DESCRIPTION OF PLATE.

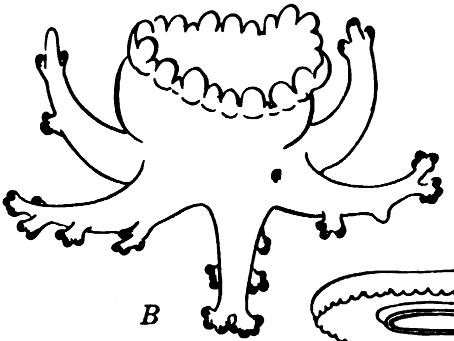
FIG. A. *Cassiopea xamachana.* The 6 medusæ have been starved for 41 days in diffuse daylight (see table 4). The recently caught, unstarved medusa in the center of the aquarium is of nearly the same size that the smallest (shown by the arrow) was when starving began. The dark-brown color of the starving medusæ is due to their densely crowded commensal plant-cells.

FIG. B. *Cassiopea xamachana* which has been starved for 41 days in darkness. Originally its bell was 25 mm. wide; now it is only 4.5 mm. in diameter, and the rim is turned upward and inward. The mouth-arms are each 3 mm. long, and the mouths have disappeared by coalescence of the lips. The medusa is still pulsating, a single rhopalium functioning.

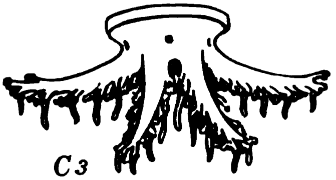
FIG. C_1. Normal medusa of *Cassiopea xamachana.*

FIG. C_2. Disk without stomach or mouth-arms.

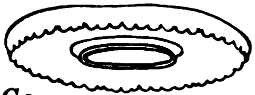
FIG. C_3. The stomach and mouth-arms of the medusa, the disk having been cut away.



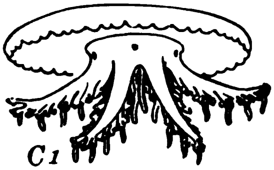
B



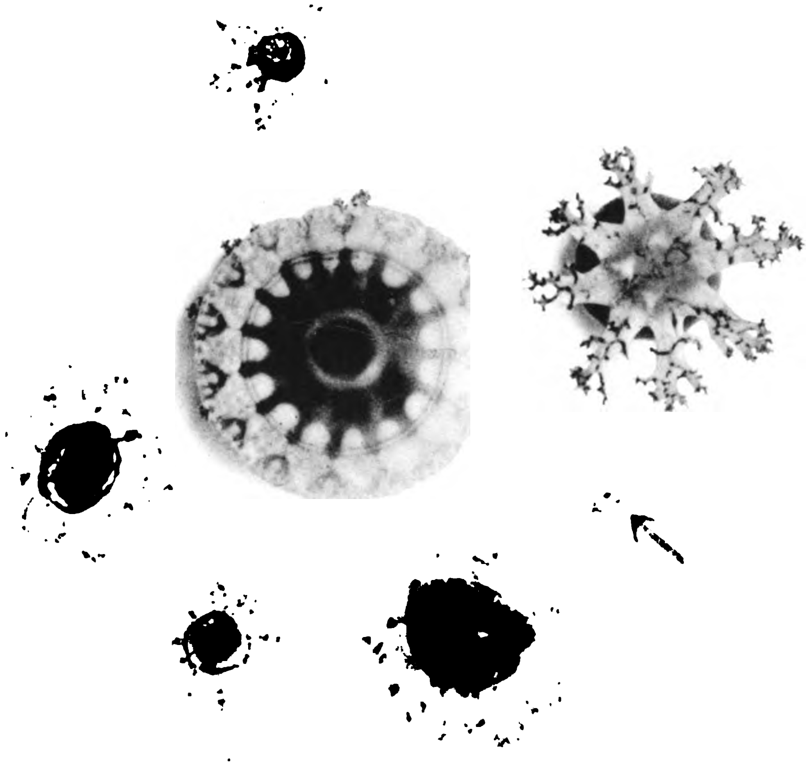
C3



C2



C1



A

IV.

CHANGES IN SALINITY AND THEIR EFFECTS UPON THE REGENERATION OF CASSIOPEA XAMACHANA.

BY A. J. GOLDFARB,
Of the College of the City of New York.

Four figures.

CHANGES IN SALINITY AND THEIR EFFECTS UPON THE REGENERATION OF CASSIOPEA XAMACHANA.

By A. J. Goldfarb.

Cassiopea xamachana, a large scyphomedusa, is very abundant in the very shallow waters of the moat at Fort Jefferson, Dry Tortugas, Florida. The conditions in this moat are such that the medusæ are in all probability subjected to extreme changes in salinity, due on the one hand to evaporation in the subtropical sun, to the shallow water and inclosed nature of the moat, to the single tide, etc., and on the other hand to extreme dilution due to the subtropical rains. Doctors A. G. Mayer and T. W. Vaughan have undertaken a study of these changes in salinity in and outside of the moat, under various conditions of tide and season. Until their report is forthcoming, this phase of the problem must be left in abeyance.

The present report considers to what extent changes in salinity influence regeneration in *Cassiopea*, and the results of the investigation are compared with those previously obtained with the hydroid *Eudendrium ramosum* of Woods Hole, Massachusetts, and with the observations of Loeb with the hydroid *Tubularia* of Serino Bay, Italy.

Though certain variable factors are known to accelerate, retard, or modify the regeneration of an organism, these factors have not heretofore been sufficiently taken into account. In the present investigation much care was taken to exclude or make the following factors uniform:

1. *Size of medusæ.* Size roughly indicates age, and as the rate of growth varies with age the medusæ finally chosen were approximately of the same size. Each solution contained one of medium size, 100 to 105 mm. in diameter, and one smaller medusa, 85 to 90 mm. in diameter.

2. *Volume, surface, and depth of solution.* To insure uniformity of respiration and evaporation, and to prevent excessive concentration, the medusæ were kept in jars of equal size and shape, each jar containing 2,000 c.c. of the solution.

3. *Aeration and change of solution.* All solutions were shaken thoroughly to aerate them, and were changed daily to avoid excess CO_2, excess evaporation, and bacteria.

4. *Degree of injury.* It has been repeatedly urged that the rate of regeneration is influenced by the extent of injury. Though recent evidence is opposed to this view, its possibility was taken into account by removing the same number of arms from each pair of medusæ.

5. *Level of amputation* has repeatedly been shown to affect considerably the rate and nature of the regenerated organ. In these experiments all arms were amputated at the same level, namely, at their respective bases.

6. *Temperature.* By avoiding local heat and local drafts the temperature was made uniform for the series.

7. *Preparation of the solutions.* To avoid disturbing the "protective relation" of the salts and to avoid introducing any salt not normally present in sea-water, the solutions were made as follows: The dilute solutions were made by adding enough (rain) water to make solutions containing 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, and 40 per cent of sea-water. The rain-water used in this series was tested by Dr. A. G. Mayer, who, upon the basis of the chlorine content, calculated that it contained 0.001 part of sea-water, an amount entirely negligible, as shown by these and other experiments. The concentrated series was made by slow evaporation in the sun's heat. In this manner, solutions containing 5, 11, 17, 25, 33, 42, and 53 per cent more than the normal salts in sea-water were obtained.

8. *The number of arms amputated* totaled 323.

9. *Three sets of measurements were recorded,* 14, 24, and 30 days after amputation. These measurements are given in table 1.

TABLE 1.—*The influence of changes in salinity on regeneration.*

Per cent of sea-water in solution.	No. of amputated arms.	Average regeneration in mm.			Remarks.
		14 days.	24 days.	30 days.	
153	15	0	0	0	
142	15	0	0	0.12	Regeneration abnormal.
133	15	0	0	0	
125	14	0.16	0	0.66	
117	15	0.21	0.62	0.43	
111	15	4.32	4.23	1.92	
105	14	3.72	3.00	2.19	Regeneration normal.
100	43	3.41	6.44	4.82	
95	15	5.46	8.41	5.69	
90	15	3.60	7.22	5.00	
85	15	4.60	7.17	5.05	
80	13	3.02	5.33	3.45	
75	15	1.87	6.09	3.74	
70	15	2.22	5.41	3.63	Regeneration abnormal.
65	15	1.26	3.64	3.33	
60	14	0.87	2.06	2.20	
55	15	0.44	1.57	2.87	
50	15	0.50	0	0	
45	15	0	0	0	
40	15	0	0	0	

The data are plotted in figure 1, in which the abscissas represent the gradient solutions and the ordinates represent the average regeneration for each solution.

From table 1 and figure 1, it will be observed that *Cassiopea* lived in solutions in which the salt content varied from a lower limit of 40 to an upper limit of 153 per cent. In the extreme solutions the medusæ decreased in size, ceased to pulsate, and the organs degenerated. This occurred in the 40 and later in the 45 per cent as well as the 153 per cent solutions.

Within these limits the medusæ lived and pulsated, but not all regenerated. No regeneration occurred in solutions containing 50 per cent or less sea-water, nor on the other hand was there any regeneration in solutions 133 or more per cent, with one exception to be noted later. Though regeneration occurred within these limitations, the character of the resulting regenerated arms was not the same. Normal pyramid-shaped arms with characteristic mouths and oral filaments were developed in the median solutions only, *i. e.*, 75 to 105 per cent inclusive. In the 70 per cent solution the mouths were considerably reduced in number, while the number of oral filaments was scarcely affected. In the 65 per cent solution there were very few mouths differentiated. In the 60 and 55 per cent solutions practically no mouths were formed and the oral filaments tended to clump together. Similarly, beginning with the 111 per cent solution, of the concentrated series, the arms were increasingly atypic. In this solution some arms were normal, others clumped as in the diluted solutions. In the 117 per cent solution, fewer mouths were differentiated and greater crowding of the oral filaments occurred. In the 125 per cent solution no mouths and only very few filaments were differentiated. It is then clear that while regeneration took place in a wide range of solutions, normal regeneration occurred within much narrower limits, namely, 75 to 105 per cent solutions.

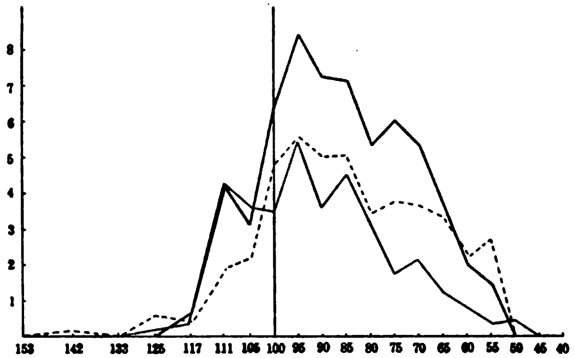


Fig. 1.—Thin line represents regeneration after 14 days: thick line 24 days; broken line 30 days.

Corroborative results were obtained from measurement of regenerated arms. Figure 1 and table 1 clearly show that when the average regeneration in sea-water is taken as the norm, then the average regeneration in 111 to 80 per cent solutions is norm or supernorm, varying from 3.4 to 5.4 mm. On either side of these limits there is a rapid decline, sharper in the more concentrated solutions, far less rapid in the more dilute series. For example, the average regeneration in the 117 per cent solution is less than in the 50 per cent solution.

The second series of measurements was taken 10 days after the first, *i. e.*, 24 days after removal of the arms. During these 10 days practically no regeneration occurred in the concentrated solutions, while retrogression set in. In all the dilute series, however, considerable regeneration occurred. This is seen in figure 1. Perhaps the facts are made clearer by table 2, which gives the difference in the average regeneration between the fourteenth and twenty-fourth days. Table 2 shows that in the concentrated solutions, 105 to 125 per cent, there was no additional regeneration; in fact, three out of four solutions showed a decrease and in one solution the increase was only 0.63 mm. In all the dilute solutions, however, there was an increase, in sea-water 3.03 mm., in 95 per cent solution 2.96 mm., 90 per cent solution 2.63 mm., etc. It should be noted that the increment was unequal, a greater relative and absolute increase occurring not in the optimum solutions, but in 75, 70, and 65 per cent solutions.

TABLE 2.—*Extent of regeneration during the second period, i. e., between the fourteenth and twenty-fourth days after amputation.*

Solutions.	Average gain or loss between 14th and 24th day.
	mm.
1.33	0
1.25	−0.33
1.17	+0.63
1.11	−0.09
1.05	−0.72
1.00 norm	+3.03
0.95	+2.96
0.90	+2.63
0.85	+2.59
0.80	+2.31
0.75	+4.22
0.70	+3.20
0.65	+2.39
0.60	+1.19
0.55	+1.14
0.50	0

In the third set of observations, made on the thirtieth day, the measurements were not taken from the amputated level, for at this stage of development it was difficult to tell with exactness where that level was. The last measurements were made from the end of the knob-like mass at the distal end of the amputated arms, and the measurements in table 1, therefore, appear smaller than the corresponding measurements for the preceding periods.

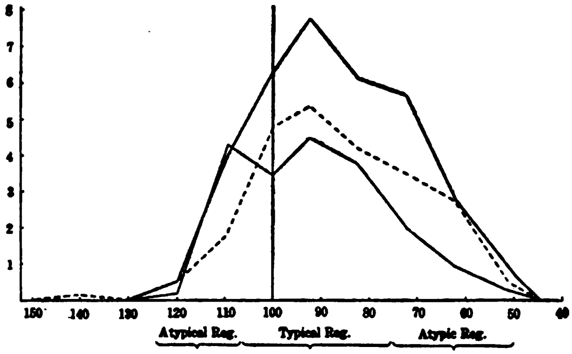


FIG. 2.—Thin line represents regenerating after 14 days; thick line after 24 days; broken line after 30 days.

The character of the curve is essentially the same as the 24-day curve, differing from it in the same way that it differs from the 14-day curve. It is seen that the curves for 105 to 75 per cent solutions are almost parallel. Beyond these limits the curves diverge. In the 75 to 55 per cent solutions there has been either an absolute or relatively greater increment than in the solutions approaching the norm. In the hypertonic solution there is no increase, the curves for the three periods are essentially parallel and overlap.

TABLE 3.

Per cent of sea-water in solution.	Average regeneration for 30 days.
	mm.
1.50	0
1.40	0.13
1.30	0
1.20	0.50
1.10	1.91
1.00	4.81
0.90	5.34
0.80	4.25
0.70	3.68
0.60	2.77
0.50	1.43
0.40	0

All these facts are made extremely clear after reducing some of the irregularities of the curves, by grouping the results on a basis of 10 per cent changes in salinity instead of 5 per cent, as shown in figure 2.

Table 3 shows the results of table 1 grouped on a basis of 10 per cent changes in salinity, instead of 5 per cent differences.

In the preceding tables and curves the fact was ignored that in certain solutions an arm was replaced by two arms. The supernumerary arms appeared in the median solutions, *viz*, 111 to 85 per cent, with a few desultory ones in more dilute solutions. The greatest number occurred in the 95 per cent solution, fewer in the 90 and 85 per cent, a sharp drop in 105 per cent and 111 per cent solutions, as shown in table 4.

Table 4 gives the number of supernumerary arms in each solution and shows that they tend to appear in optimum dilute solutions.

TABLE 4.

Per cent of sea-water in solution.	No. of arms removed.	No. of double arms regenerated.	Per cent of double arms.	Per cent of sea-water in solution.	No. of arms removed.	No. of double arms regenerated.	Per cent of double arms.
1.53	15	0	0	85	15	4	26
1.42	15	0	0	80	13	0	0
1.33	15	0	0	75	15	0	0
1.25	14	0	0	70	15	0	0
1.17	15	0	0	65	15	2	13
111	15	1	7	60	14	0	0
105	14	1	7	55	15	1	7
100	43	5	11	50	15	0	0
95	15	5	33	45	15	0	0
90	15	4	26	40	15	0	0

When the supernumerary arms are included in table 1, it is found that the highest part of the curve, 85 to 111 per cent, is augmented still further. The maximum augmentation occurs in 85 to 95 per cent solutions, and only a moderate increment occurs in sea-water.

Whether we consider only the regeneration of the supernumerary arms or of single arms, or normal as opposed to abnormal regeneration, or the variation in growth at different intervals, or all of these combined, it is clear that the maximum regeneration in *Cassiopea* occurs in sea-water diluted to 95, 90, and 85 per cent.

There are three sets of records complete enough to permit comparison, namely, Loeb's work on *Tubularia*,[1] Goldfarb on *Eudendrium*,[2] and the present contribution on *Cassiopea*.

Loeb's data are based upon the regeneration of 65 pieces of *Tubularia* stems over a period of 8 days. The work was done at Serino where the sea-water is estimated on the basis of Forchhammer's calculation to contain 3.8 per cent salts or more correctly 38.364 per kilogram. Loeb used 10 grades of solutions and plotted his results as shown in figure 3.

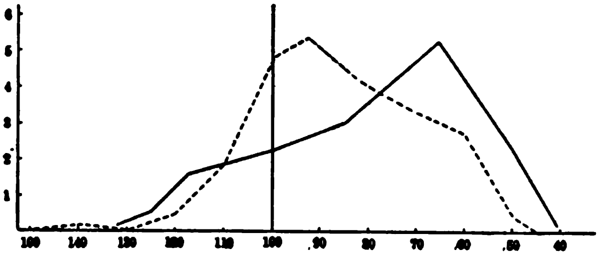


FIG. 3.—Thick line is Loeb's curve for *Tubularia*; broken line is curve for *Cassiopea* after 30 day interval.

The 323 *Cassiopea* arms observed at 14, 24, and 30 day intervals in sea-water whose salinity, though not definitely known, is calculated[3] to be 3.549 per cent. The medusæ were placed in 20 graded solutions. The results differ from Loeb's in at least four respects:

1. The salinity limits are different, *i. e.*, *Tubularia* regenerated in more dilute solutions than *Cassiopea*, though the extreme concentration is probably the same for both.

2. Maximum regeneration occurs at different salinities, 65 per cent sea-water for *Tubularia*, 95 per cent for *Cassiopea*.

3. There is a greater range of supernormal regeneration in *Tubularia* *i. e.*, 100 to 50 per cent salinity, while the range for *Cassiopea* is limited to 15 per cent, *i. e.*, from norm to 85.

4. The character of the curves is reversed. For *Tubularia* there is a graded increased regeneration with increasing dilution from 133 to 65 per cent; beyond this limit there is a sudden drop to complete inhibition in 40 per cent. The *Cassiopea* curve on the other hand rises rapidly to 95 per cent, beyond which there is a very gradual decrease (fig. 3).

The third set of observations were those on the compound hydroid *Eudendrium ramosum*. The results of one series, based on 953 stems, are plotted in figure 4, and when compared with *Tubularia* and *Cassiopea* show the following:

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[1] Loeb, J. Organization and growth. Wurtzburg. 1891.

[2] Goldfarb, A. J. Factors in the regeneration of a compound hydroid, *Eudendrium ramosum*. Jour. Exper. Zool., vol. 4, p. 43. 1907.

[3] Clarke, F. W. The data of geochemistry. Bull. 491, U. S. Geol. Survey. 1911.

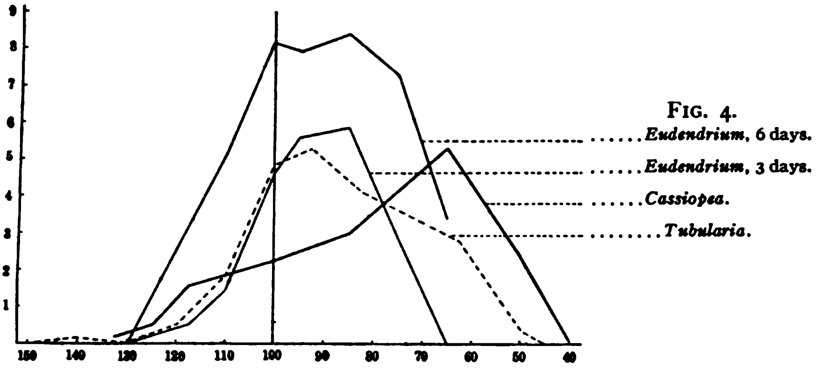

FIG. 4.

1. Regeneration takes place in a more limited variation from the norm than either *Tubularia* or *Cassiopea*, *i. e.*, the maximum salinity in which regeneration takes place is 120, the minimum is 60.

2. Maximum regeneration is intermediate, 65 per cent solution for *Tubularia*, 85 for *Eudendrium*, and 95 for *Cassiopea*.

3. Normal or supernormal regeneration in *Tubularia* occurs over a wide range of solutions, from norm to 50 per cent; in *Eudendrium* it is intermediate, 100 to 80 per cent; in *Cassiopea* most limited, 100 to 85 per cent.

4. The nature of the curves varies. *Tubularia* and *Cassiopea* have asymmetrical curves, *Eudendrium* almost symmetrical, *i. e.*, beyond the optimum solution, increased dilution or concentration is equally injurious.

Two sets of observations after 3 and 6 day intervals afforded the opportunity of comparing these with corresponding sets of observations on *Cassiopea*. The regeneration in *Eudendrium* is given in table 5.

TABLE 5.

Per cent of sea-water in solution.	Number of polyps regenerated in—		
	First 3 days.	Second 3 days.	Total, 6 days.
130	0	0	0
117	5	27	32
111	14	37	51
100 (norm)	46	36	82
95	56	23	79
85	62	22	84
75	28	45	73
65	0	34	34

It is apparent that the same phenomenon observed in *Cassiopea* occurred in *Eudendrium*, namely, that with increased intervals there is a greater relative regeneration, not in the optimum nor hypertonic solutions, but in the more dilute solutions, such as 75 and 65 solutions. It is very clear, both from *Cassiopea* and *Eudendrium*, that such changes in the sub-optimum solutions do not and can not alter the essential character of the curves sufficiently to make it analogous with Loeb's. The differences in the behavior of the three organisms is made easier for comparison by table 6, which gives the results of changes in salinity upon the three organisms studied by Loeb and Goldfarb.

TABLE 6.

	Maximum dilution.	Maximum concentration.	Optimum solution.	Regeneration occurs in—	Beyond optimum on concentrated side of curve.	Beyond optimum on diluted side of curve.	Salinity.	Curve.
	p. ct.	p. ct.	p. ct.	p. ct.				
Tubularia . . .	40	133	65	100 to 50	Less injurious.	Most injurious.	3.83	Asymmetrical.
Cassiopea . . .	50	133	95	100 85	Most injurious.	Least injurious.	3.54	Less asymmetrical.
Eudendrium .	60	125	85	100 80	Equally injurious.	Equally injurious.	3.29	Symmetrical.

In these three organisms the increasing dilution of sea-water, with the consequent intake of water into the organism, makes for a more rapid and greater growth. Beyond an optimum dilution, any further dilution retards and ultimately inhibits regeneration. Concentration of sea-water, in all cases, makes for an expulsion of water from the organism, with a concomitant retardation, decreased regeneration, and finally no regeneration whatsoever. Beyond these fundamental similarities, there is little or no agreement.

Though there appears to be little or no similarity in the behavior of these three organisms, on further examination it becomes quite clear that *Eudendrium* and *Cassiopea* are in essential agreement, both differing in a striking manner from Loeb's results on *Tubularia.*

Eudendrium and *Cassiopea* agree in the following respects: (1) maximum regeneration occurs in solutions that are hypotonic in a relatively small degree, *i. e.*, 95 and 85 per cent respectively; (2) regeneration in sea-water is equaled or exceeded in a narrow range of hypotonic solutions, *i. e.*, 100 to 85 and 80 per cent, respectively; (3) at any interval after amputation, the graphs show a *sharp* decline in the amount regenerated in increasingly hypertonic solutions; a maximum regeneration of not more than 60 per cent beyond the norm, *i. e.*, 60 per cent in *Cassiopea*, 35 per cent in *Eudendrium;* a *gradual* followed by a more rapid drop in the curve, in solutions more hypotonic than these optimum solutions.

It is in these very points that both these curves differ from Loeb's work on *Tubularia*, as follows: The maximum regeneration occurred in a markedly greater hypotonic solution, *i. e.*, 65 per cent; regeneration in sea-water is equaled or exceeded in a considerably wider range of diluted solutions, *i. e.*, 100 to 50 per cent solutions inclusive; the curve is completely reversed, the gradual increment and the sharp decline appearing on sides opposite to that in either *Eudendrium* or *Cassiopea;* the mode is nearer the extreme dilution; and the maximum regeneration is far greater (120 per cent) than in either of the other two organisms.

It is estimated that the maximum salinity of the sea-water at Woods Hole, Massachusetts, is 3.29,[1] for the Dry Tortugas 3.54 per cent.[2] It might be urged that a definite relation obtains between the density of the sea and the regeneration of the contained organism, subjected to a graded series of densities, for the increased density of the sea-water at the Dry Tortugas is paralleled by each of the changes already cited in table 6; but when *Tubularia* of Serino Bay, whose density is 3.83, is taken into consideration, no correlation between density and the character of the curve obtains, except in two particulars: (1) the greater the density of the sea, the greater the dilution in which regeneration will take place; (2) the greater the density of the sea the greater the regeneration in the hypotonic solutions, *i. e.*, *Eudendrium* Δ 3.29, maximum regeneration is 35 per cent above norm *Cassiopea*, Δ 3.54, 60 per cent, *Tubularia*, Δ 3.83, 120 per cent.

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[1] Bulletin of Bureau of Fisheries, vol. 31. 1911.

[2] Clarke, F. W. The Data of Geochemistry. Bull. 491, U. S. Geol. Survey. 1911.

Subject to a more accurate analysis of the density of the water in the moat at Fort Jefferson and at Serino Bay, these conclusions must be considered tentative only. The fact remains, however, that Loeb's limited data are not in accord with the results obtained in two different organisms from two different localities whose sea-water was of different densities. It is also clear that the more detailed and more numerous data upon *Eudendrium* and *Cassiopea* are in close agreement. And we are driven to the conclusion that either *Tubularia*, for some inexplicable reason, differs in its behavior from the other two cœlenterates, a view which is very improbable, or that the insufficient data upon *Tubularia* has misled the author. The close similarity of *Eudendrium* and *Cassiopea* and the corroborative results of A. G. Mayer upon the effects of changes in density upon nerve-conduction leads me to believe that the results here given may be taken as indicative of the gross effects, without any effort to segregate the osmotic from the ionic influences, upon the regeneration of these organisms.

SUMMARY.

The object of this investigation was to ascertain to what extent changes in salinity affected an organism, *Cassiopea xamachana*, normally subject to relatively great variation in the concentration of the sea-water, and to compare the results with those of the hydroid *Eudendrium* and the hydroid *Tubularia*.

Considerable attention was paid to render the following variable factors uniform for the series: Size of medusæ; volume, surface, and depth of the solutions; extent of injury; level of amputation; temperature; crowding.

Injurious or other variable factors were guarded against: (1) by aerating and changing all solutions daily; (2) by diluting with water containing known amount of sea-salts; (3) by concentrating the sea-water very slowly; (4) by examining the 323 regenerating arms at intervals of 14, 24, and 30 days.

Cassiopea lived in solutions ranging from 40 to 153 per cent sea-water solutions.

Regeneration occurred in solutions containing 50 to 133 per cent sea-water.

Normal regeneration of the arms occurred within much narrower range, namely, 75 to 105 per cent. Beyond these limits regeneration was atypic.

While the amount regenerated in sea-water was equaled or exceeded in solutions diluted to as much as 85 per cent, the optimum solution was 95 per cent sea-water. With increasing concentration, regeneration fell off rapidly; with increasing dilution the amount regenerated was diminished very slowly.

During the succeeding 10 and 16 days there was a relatively greater regeneration in solutions below the optimum, *i. e.*, in the 75 and 70 per cent, and the character of the curve was correspondingly altered.

The development of supernumerary arms likewise occurred most frequently in the optimum solutions, namely, 95 to 85 per cent; less frequently with increasing dilution or concentration.

When these results are compared with those of Loeb on *Tubularia* and with the writer's on *Eudendrium*, it is clear that in all the maximum regeneration does not take place in sea-water, but in sea-water diluted, and that with increasing concentration or dilution regeneration is retarded and diminished.

The character of the curves representing the influence of changes in salinity upon the regeneration of these organisms is strikingly different.

1. The most dilute solutions in which regeneration will take place is 40 per cent for *Tubularia*, 50 per cent for *Cassiopea*, and 60 per cent for *Eudendrium*.
2. The maximum regeneration occurs in 95 per cent solutions for *Cassiopea*, while it is 85 per cent for *Eudendrium* and 65 per cent for *Tubularia*.
3. The regeneration in sea-water is equaled or exceeded in solutions diluted as much as 13 per cent for *Cassiopea*, 20 per cent for *Eudendrium*, and 50 per cent for *Tubularia*.
4. The curve is most asymmetrical for *Tubularia*, less so for *Cassiopea*, and almost symmetrical for *Eudendrium*.
5. The *Eudendrium* behaves more like *Cassiopea;* both are strikingly different from *Tubularia*.

It is very evident that Loeb's curve is not representative of the behavior of marine organisms to changes in concentration, and it is altogether probable that such a curve can not be expressed in a simple curve based on two variables.

In the absence of information concerning the exact concentration of the sea-water, where each of these experiments was made, it is unprofitable to make extended generalizations.

V.

REGENERATION IN THE ANNELID WORM AMPHINOMA PACIFICA, AFTER REMOVAL OF THE CENTRAL NERVOUS SYSTEM.

By A. J. GOLDFARB,
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REGENERATION IN THE ANNELID WORM AMPHINOMA PACIFICA, AFTER REMOVAL OF THE CENTRAL NERVOUS SYSTEM.

BY A. J. GOLDFARB.

In a previous publication,[1] the writer found that the head of the earthworm *Lumbricus* was regenerated in the entire and permanent absence of the nerve-cord from the amputated region. Inasmuch as this was the first demonstrative experiment of a complete and perfect regeneration in the absence of the nerve-cord, it seemed extremely important to ascertain whether the phenomenon was peculiar to *Lumbricus*, and therefore of limited significance, or whether other annelid worms could likewise regenerate without the aid or presence of the nerve-cord. With this object in view, the marine annelid *Amphinoma pacifica* was chosen, because it lives so well in the laboratory, because large numbers could be readily procured, but especially, because it was sufficiently large and hardy to permit of the necessary operations.

There were two types of operations:

1. After narcotizing in a 5 per cent solution of alcohol in sea-water, the worms were amputated and the nerve-cord extracted. This was done by passing a fine forceps into the cœlomic cavity in such a manner that the nerve-cord lay between the tongs of the forceps, and at any desired position the forceps were firmly brought together and the contained nerve-cord was extracted with its ganglia and bases of the nerves. These could then be readily counted. I never succeeded in getting pieces so long as those in the earthworm, illustrated in my report.[1] I did succeed in extracting as many as nine consecutive ganglia. This method had the distinct advantage of causing very little injury to adjoining tissues. The importance of this fact will be later emphasized.

2. The second type of operation, used by Morgan[2] and modified by Nussbaum,[3] consisted in cutting a "window" from the ventral side near the amputated level. The parts so removed or injured included cuticle, muscle layers, nerve-cord, often the ventral blood-vessel, and the nephridia. A modification of the operation consisted in making a ventral incision, then

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[1] Goldfarb, A. J. The influence of the nervous system in regeneration. Jour. Exp. Zool., 7, 4, pp. 643–722. 1909.

[2] Morgan, T. H. Experimental studies of the internal factors of regeneration in the earthworm. Arch fur Entw. Mech., 14. 1902.

[3] Nussbaum, J. Beitrage zur Frage über die Abhängigkeit der Regeneration vom nervensystem bei *Nereis diversicolor*. Arch. f. Entw., vol. 25. 1908.

deflecting the muscle layers sufficiently to permit the removal of the exposed nerve-cord. The muscle walls were then replaced and ligatured.

There were 90 operations of the first type, 12 of the second, and 151 worms served as controls.

The worms were amputated at different levels and the nerve-cord removed from the anterior as well as the posterior parts. It is well known that the regeneration from the two exposed surfaces at certain levels may be quite different. The flatworm *Dendrocœlum lacteum*,[1] for example, regenerated a tail from the anterior piece and a head from the posterior piece, provided the amputation was not made more than one-third from the anterior end. Posterior to this level the head piece regenerated a tail, but the tail piece did not regenerate a head. In the earthworm *Lumbricus* the same phenomenon occurs. The differentiating level occurs at about the fifteenth segment.

Amphinoma differs from this type of regeneration in two respects: (1) the level at which the two cut surfaces can regenerate is much more posterior, *i. e.*, about the middle of the body; (2) instead of the regenerative power of the tail pieces being limited to certain narrowly prescribed levels, the reverse obtains, *i. e.*, head pieces can regenerate tails only in the posterior half or third of the worm, while tail pieces regenerate heads at practically all levels.

Table 1 gives some details of the regeneration in the head pieces of control worms, *i. e.*, level amputated, the number that regenerated a tail, etc. It is evident that no head piece regenerated when amputated less than one-eighth from the anterior end of the worm. Between the third and the middle of the body some of the pieces regenerated, others did not. All, however, regenerated posterior to the forty-fifth segment or middle of the body.

TABLE 1.

Level amputated.	No. of worms.	No. survived.	No. regenerated.	No. with no regeneration.
One-eighth from anterior end....	9	8	0	8
Twenty-fifth segment..........	1	1	1	0
Thirty-fifth segment...........	1	1	1	0
One-third from anterior end.....	3	3	2	1
Middle of worm...............	5	5	2	3
Forty-fifth segment and farther posterior by actual count.....	9	9	9	0
Three-fourths from anterior end.	4	4	4	0

In table 2 similar data are given for tail pieces of control worms. The data are arranged with respect to the level amputated and the number that did or did not regenerate. All of the tail pieces regenerated a head from the first quarter, the middle, and even from the distal quarter of the worm. Beyond this level a number of pieces did not regenerate. There were 9 such pieces that formed no head. Of these, 5 were small distal pieces 12 to 17 segments from the distal end, and probably too small to regenerate; in the other 4, I have no records of the exact level amputated.

TABLE 2.

Level amputated.	No. of pieces.	No. survived.	No. regenerated.	No. that did not regenerate.
One-fourth to middle..........	10	3	3	0
One-eighth to middle..........	22	13	13	0
One-eighth to seven-eighths.....	10	8	8	0
One-fourth to one-half.........	(?)	19	19	0
One-eighth to seven-eighths.....	18	17	10	7
One-eighth to seven-eighths.....	13	11	9	2

Tables 1 and 2 establish the fact that at anterior levels the head piece can not regenerate a tail, while the tail piece can regenerate its head. At posterior levels the reverse occurs: the tail piece can not regenerate a head, though the head piece can regenerate a tail. In the median levels both pieces may regenerate their respective missing parts. These preliminary facts concerning the regeneration of *Amphinoma*, after ordinary amputation, will aid in understanding the behavior of the head and tail pieces after the removal of the nerve-cord as already described. Of the 56 operated head pieces, 29 survived 25 to 30 days, as shown in table 3, which also gives the level amputated and the number that failed to regenerate.

TABLE 3.

	No.	No. regenerating a tail.
Operated head pieces......................	56	....
Survived................................	29	....
Amputated at one-eighth level............	8	0
one-fourth level............	3	0
one-third level.............	10	0
one-half level..............	7	0
three-fourths level..........	1	0

These operations were made at anterior levels before it was known that head pieces did not normally regenerate from these parts of the worm. Though regeneration took place readily at posterior levels of control worms, not one operated head piece developed a tail. The experiment appears to demonstrate that, in these instances at least, regeneration had been prevented by destroying the nerve-cord near the amputated level.

I have elsewhere suggested[1] that regeneration could be prevented and frequently was prevented by various means other than injury to the central nervous system. In the newt *Diemictylus*, the regeneration of the tail was prevented by plugging the cavity of the spinal column with paraffin. In the salamander Tornier[2] prevented the formation of the tail by operating the skin and muscle tissues in a manner to prevent their coordinate development. In *Amphinoma* regeneration was prevented not only by injury to the nerve-cord, but by removing the alimentary tract from five or more segments nearest the amputated level. In table 4 the anterior pieces with the alimentary tract so removed are arranged according to the level amputated.

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[1] Goldfarb, A. J. The influence of the nervous system in regeneration. Jour. Exp. Zool., vol. 7, 1909. The central nervous system in relation to the phenomenon of regeneration. Arch. f. Entw., vol. 32, 4. 1911.

[2] Tornier, G. Kamp der Gewebe im Regenerat bei Begunstigung der Hautregeneration. Arch. f. Entw,, 22, 1906, p. 348.

TABLE 4.

Number of worms.	Number survived.	Level amputated.	Number regenerated.	Number not regenerated.
3	2	One-eighth	0	2
3	2	One-third	0	2
3	2	One-half	0	2
3	2	Three-fourths....	1	1

That regeneration was not prevented by injury to, or absence of, the nerve-cord can be demonstrated only by more direct proof. It must be shown (1) that regeneration did not take place, even though the amputated region was re-innervated by the regenerated nerve-cord; (2) that regeneration could take place without any trace of the nerve-cord in the region adjoining the amputated level. This proof was obtained by a detailed examination of serial longitudinal sections of posterior pieces. Table 5 includes the 46 posterior pieces from which the nerve-cord had been removed and which were subsequently preserved 25 to 30 days later.[1]

TABLE 5.

Amputated level.	Regenerated.	No regeneration.
One-eighth............	5	11
One-fourth............	0	3
One-third.............	0	3
One-half..............	2	3
Three-fourths..........	0	1

Out of 28 worms, 7 regenerated a distinct head; the other 21 did not regenerate in the same interval. Worm number 33 is typical of the 7 operated worms which regenerated their heads. The serial sections showed that the nerve-cord had been completely removed from the seven segments nearest the amputated level, agreeing with the record made at the time of operation. It was found that typical ganglia of the nerve-cord extended as far as the middle of the sixth segment from the amputated level, at which point the nerve-cord suddenly became narrow and extended in this very narrow, partially ganglionated condition into the fifth, fourth, third, second, and first segments, beyond the amputated level into the regenerated head. It continued dorsally and ended finally in a clearly differentiated supra-œsophageal ganglion. The details are identical with those described elsewhere[2] for *Lumbricus*. These worms afford no clue to the dependence or independence of regeneration upon the presence of the nerve-cord at the amputated level, nor of the manner in which the central nerve system was formed.

The second group of operated worms did not regenerate the head. I have arbitrarily designated a regenerated head one in which at least the stomadeum and "brain" were clearly differentiated. Worm number 32 may serve as an example of this group. In this worm the characteristic

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[1] The high mortality occurred chiefly in worms operated by the "window" method of removing the nerve-cord.
[2] Goldfarb, A. J., *vide*.

nerve-cord was present as far as the eighth segment from the amputated level. Beginning at the seventh segment the nerve-cord was very narrow and extended in this attenuated condition through the sixth, fifth, fourth, third, second, and first segments. The regenerated nerve-cord had not only reached the amputated level, but extended slightly beyond, into the proliferated embryonic tissues laid down at the cut end. A similar regeneration of the nerve-cord occurred in worms 13, 9, 45, etc., in which the nerve-cord regenerated two to seven or more segments, and in which the nerve-cord did not always reach or pass beyond the amputated level.

In the worms whose nerve-cords had regenerated to the amputated level, supplying this region with nervous and trophic stimuli, the lack of regeneration could not have been due to any lack of innervation.

In a third very small group the head regenerated without the presence of the nerve-cord at the amputated level. In worm No. 7, for example, the characteristic nerve-cord extended as far as the sixth segment from the amputated level. The regenerated nerve-cord was present in the fifth and part of the fourth segment. Beyond this region, *i. e.*, the fourth, the third, the second, and posterior half of the first segment, there was no trace of a nerve-cord. Close to the amputated level and slightly beyond it into the embryonic tissues, there was an unmistakable ganglionic mass, from which there extended posteriorly a short, narrow nerve-commissure. This supraœsophageal ganglion had been formed independently of the direct contact or stimulation from the regenerating nerve-cord.

Worm No. 34 affords a much better idea of the development of the newly formed head. The nerve-cord was intact as far as the sixth segment, from which point the regenerated nerve-cord extended through the fifth, fourth, and third segments. The next two segments were entirely free of traces of the nerve-cord. Beyond the amputated level there was a fairly large regenerated head with a clearly differentiated "brain," from which the nerve-commissures extended ventrally and posteriorly almost to the amputated level. These two worms clearly show that it is possible for a head to regenerate with the nerve-cord 2, 3, or more segments from the amputated level. They show that the development of the brain and commissures may secondarily unite with the regenerating nerve-cord, as was found to be the case in *Lumbricus*.

In view of the regeneration of the head in *Lumbricus*, in the absence of innervation from the nerve-cord, and in view of the corroborative results obtained in *Amphinoma*, it seems evident that regeneration can take place under these circumstances. It is very probable that the presence of the nerve-cord accelerated regeneration. It may also be that regeneration does not take place ordinarily until the amputated level has been innervated from the cells of the nerve-cord as urged by Nussbaum.[1] It is equally clear, however, that a head can be regenerated in at least two annelid worms without the aid or stimulus derived from the nerve-cord.

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[1] Nussbaum, Josef. Beitrag zur Frage uber die Abhangigkeit der Regeneration vom Nervensystem be *Nereis diversicolor*. Arch. f. Entw., vol. 25. 1908.

SUMMARY.

The marine annelid worm *Amphinoma pacifica* readily regenerated a head at all levels except the distal eighth of the worm.

The formation of a tail occurs only in the posterior half or third of the body.

Only in the median levels of the body can the two cut surfaces regenerate a head and a tail respectively.

Regeneration may be prevented by a severe injury, either to the digestive tract or to the central nerve system; the greater the injury the more likely will regeneration be inhibited.

Many pieces did not regenerate after removing the alimentary tract from five or more segments nearest the amputated level.

Many pieces, about one-third, failed to regenerate after removing the nerve-cord by the forceps, *i. e.*, with little injury to adjoining tissues.

All failed to regenerate after removing the nerve-cord by the "window" method, *i. e.*, with serious injuries to adjoining parts.

The operated worms were examined in serial sections. In one group a regenerated nerve-cord connected the regenerated "brain" and commissures with the old intact nerve-cord.

In a second group the regenerated nerve-cord approached and in many instances reached the amputated level, yet no head was formed.

In a third group, the nerve-cord had regenerated, but several segments nearest the amputated end were yet without any nerve-cord or ganglia. These worms nevertheless had regenerated a head with its typical brain and nerve-commissures.

The last group completes the demonstration that *Amphinoma* as well as *Lumbricus* can regenerate without the contact of, or stimulation from, the nerve-cord or central nervous system.

VI.

EXPERIMENTALLY FUSED LARVÆ OF ECHINODERMS WITH SPECIAL REFERENCE TO THEIR SKELETONS.

By A. J. GOLDFARB,
Of the College of the City of New York.

Fifteen text-figures.

EXPERIMENTALLY FUSED LARVÆ OF ECHINODERMS WITH SPECIAL REFERENCE TO THEIR SKELETONS.

BY A. J. GOLDFARB.

The early work of Loeb, Morgan, and Herbst on the production of multiple embryos from a single egg suggested the reverse experiment of grafting or reuniting several fertilized eggs into one embryo. This important experiment was first performed by Driesch, who succeeded in grafting together two eggs in about 0.04 per cent of the culture. With the same technique he subsequently grafted the eggs in other species of echinoderms and succeeded in keeping such grafted groups alive until they had developed into the larval or pluteus stage. He found that these larvæ, fell into several distinct types, as follows: true twins, twins with a common blastocœle, twins with a reciprocal influence on growth, fusion with a partially double archenteron, perfect fusion with a single set of organs, and single body with a second parasitic archenteron.

In 1912, the writer repeated these experiments with the American form *Arbacia punctulata* and succeeded only after slightly modifying Driesch's method. In the best cultures about 40 per cent were fused in groups of 2, 3, 4 or more eggs. These fused groups were preserved at different stages of development for further detailed study, but sufficient precautions had not been taken to guard against the solution of the very delicate skeletal structures of the larvæ. Consequently the early stages only were available for detailed and complete study. Such studies corroborated and extended the results of Driesch.

Subsequently, in the performance of other experiments, it was discovered that eggs could be agglutinated and fused quite as readily by a very different method, which was not only simpler but free of certain objections that might be urged against previously known methods. The new method consisted in using an isotonic or slightly hypotonic NaCl solution diluted with varying quantities of sea-water.

NEW METHOD OF FUSING EMBRYOS.

The best results were obtained by the following procedure: The eggs of *Toxopneustes* were artificially fertilized, and 15 to 20 minutes later approximately equal quantities were placed in solutions made up of 70 to 80 parts of 5/8 m. NaCl (isotonic with sea-water at the Tortugas) and 30 to 20 parts of sea-water. After 5 to 8 hours in these solutions the eggs were transferred to fresh sea-water. In the best cultures the number of agglutinations and subsequent fusions were as numerous as in the best cultures of *Arbacia* with the modified Driesch's method.

EARLY DEVELOPMENT.

Inasmuch as I have elsewhere described in detail the early developmental changes in agglutinated and fused embryos of *Arbacia* and since the changes in *Toxopneustes* are essentially identical with those in *Arbacia*, I will only supplement the previous account by stating briefly at what stage and under what circumstances agglutination occurred after treatment with the NaCl solutions.

The addition of an isotonic NaCl solution to sea-water involves a dilution of the other sea salts and a concentration of the NaCl. The excess NaCl stimulates the eggs to develop more rapidly, provided that the solution does not contain more than 50 per cent of the NaCl solution. In solutions containing 50 to 60 per cent NaCl solution, the excess NaCl seems to be neutral, but beyond this quantity with 90 or more per cent development was distinctly retarded and atypic. That the eggs in the NaCl solutions absorbed water was evidenced by the distinct increase in size over the corresponding controls, and by the diminution in the space between the egg and the fertilization membrane. In many instances the fertilization membrane burst and a part of the protoplasm of one or more of the blastomeres protruded from the fertilization membrane, while in other eggs this membrane was completely thrown off.

The differential rate of development in the NaCl solutions closely paralleled the differential rate of regeneration in varying concentrations of sea-water. This suggested that the agglutinations might be due to a graded intake of water. But no agglutinations occurred in ordinary diluted or concentrated sea-water, or in sea-water to which $MgSo_4$, K, Ca, or Li salts had been added, or in sea-water to which various anesthetics had been added, or with eggs whose fertilization membranes had been removed, or in sea-water rendered alkaline. The NaCl in the above experiments must have affected the eggs in such a manner as to gelatinize their outer surfaces, and under these circumstances, when two or more eggs whose fertilization membranes were ruptured came in contact, they were agglutinated. Some of these agglutinated eggs subsequently separated, others remained agglutinated, developing into more or less perfect twins, while others fused together. I have never observed fusion during the egg stage, as reported by Haan. The fusion in both *Arbacia* and *Toxopneustes* occurred and was limited to the blastula and gastrula stages, as described in a previous publication. In these studies and in those of other workers in this field attention has been chiefly directed to the fusion of the ectodermal walls of the two embryos and to the fusion of their archentera. The skeleton seems to have been overlooked. Curiously enough the changes in the skeletons of two fusing larvæ indicate most clearly the nature of the regulatory phenomena during this process. This paper will deal with the consideration of the changes in the skeletal structures in fusing larvæ.

SINGLE PLUTEUS LARVA.

Except where specifically mentioned to the contrary the drawings were made with the camera lucida and drawn to the same scale. Figure 1 is a lateral view of a single larva fully devloped. The figure represents the larva somewhat foreshortened, but with all of the parts, particularly the skeletal structures, outlined. Within the body of the larva are two ventral body rods, one on each side of the body. From these rods two long bars extend into the ventral arms. At right angles to these, and arising from the union of the ventral body rods and the ventral arm rods, are two ventro-dorsal connectives, which in turn bifurcate, one branch extending into the dorsal arm and the other aborally into the body to form the dorsal body rod. From the aboral end of the ventral body rods are two usually bifurcated aboral branches. Finally, there are two ventral branches between the ventral arms, but these are not shown in the figure. These bars are easily recognized from one another in both the single and fused larvæ, and they afford a definite and measurable index of the changes during the fusion of the larvæ.

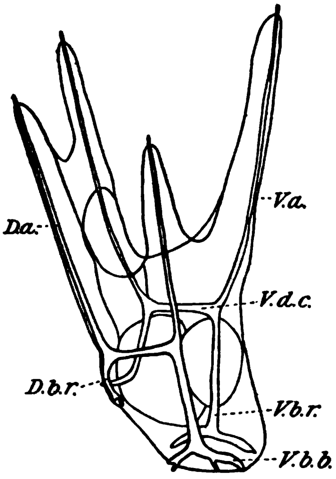

FIG. 1.

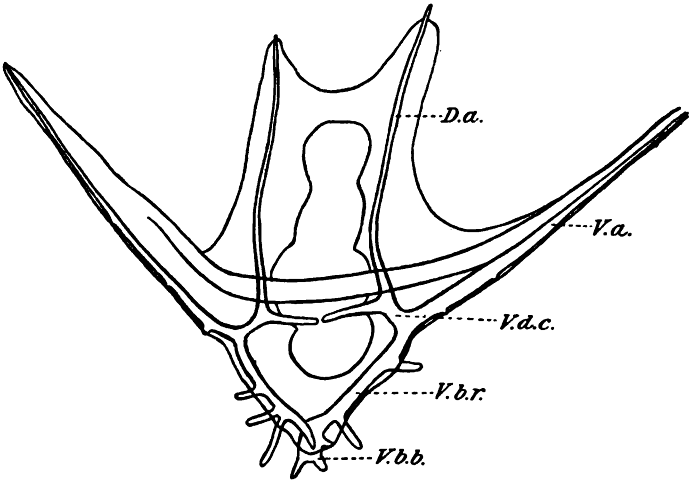

FIG. 2.

Figure 2 is another single larva drawn under greater magnification, It shows, from a foreshortened ventral view, the same characteristic parts of the skeleton, and also illustrates the small knobs and processes on the ventral rods, which are sometimes not reproduced in the drawings. Figures 3 and 4 are enlarged to the same scale as figure 2 and represent the ventral and lateral views of the skeleton. Figure 4 affords a particularly good example of the spinous ventral body rods.

FUSED PLUTEI.

Agglutinated or "true twin" larvæ are of no special significance for my present purpose, for each is perfectly developed and no reciprocal influence occurred. Figures 1 to 3 correctly represent any such agglutinated larva. It is only in fused larvæ, *i. e.*, larvæ with a common body cavity and a common and continuous ectodermal wall, that reciprocal changes are observed.

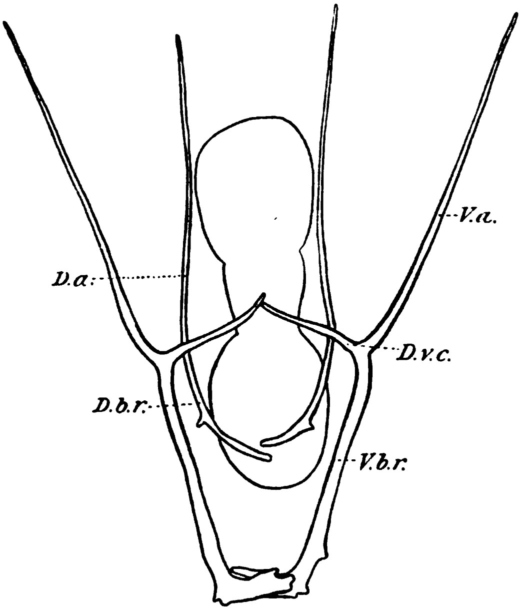

FIG. 3.

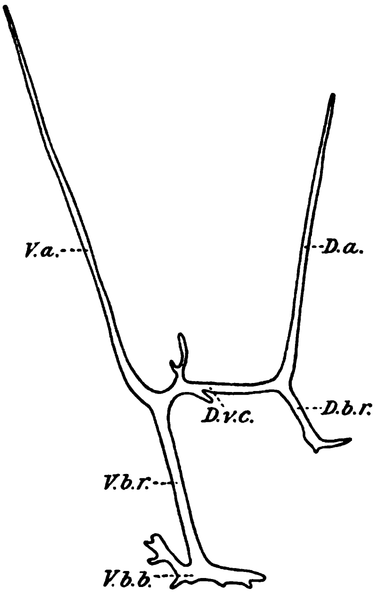

FIG. 4.

In the following drawings, for the sake of simplicity, I have not always indicated the whole skeleton when such omitted portions are normal and when the symmetrical right or left side is fully represented. Figure 5 is a partial fusion of two larvæ with a common body cavity and a continuous body wall. Each larva is completely differentiated and normal. The archentera are complete and independent, though they appear to be superimposed in this particular view of the fusion. The skeletons are typical and the parts are complete and characteristic of the normal single larva. On closer examination it is found that certain parts have not developed or have not reached their full size, such as the left dorsal rods of the right or A pluteus, and the left dorsal arm of the left or B pluteus. Associated with this suppression or retardation of the dorsal rods there is a compensatory growth of the left dorsal arm of the B pluteus. This supernumerary arm bifurcates, one branch extending into the right pluteus, the other branch into the left pluteus. And though the skeletal rods of the two larvæ are in close proximity and overlap in several places, there is no trace of a fusion of the rods. This suppression of the dorsal rods and the associated compensatory growth of other parts of the skeleton were observed in varying degrees of complexity in all or nearly all the fused larvæ.

The two plutei of figure 6 are fused more completely together than the preceding pair of plutei. The two larvæ are perfect and the parts are typical and independent, except the body wall, which is common to both. In the right or A pluteus each of the skeletal parts is differentiated and normal. In the left or B pluteus the ventral body rods are incomplete in so far as they do not possess their aboral bifurcated branches, and one of the ventral arm rods is abbreviated. The B pluteus in this fusion is incomplete, not with respect to the dorsal rods, as in the previous example, but in the aboral branches of the ventral body rods.

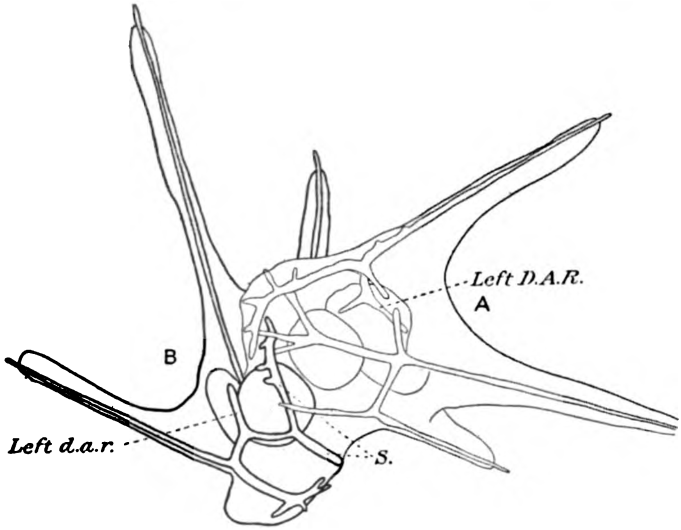

FIG. 5.

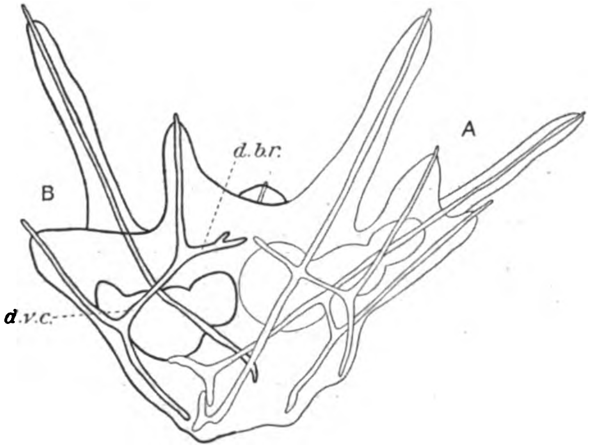

FIG. 6.

It is well known that in the development of the skeleton the mesenchyme cells differentiate two triradiate spicules, one on each side of the body. The bars of the triradiate spicules develop unequally. The ventral body rod, the ventral arm rod, and the dorso-ventral connective are formed from the outgrowth of the original three bars. Subsequently the dorsal body rod and the dorsal arm rod are differentiated, and finally the aboral branches of the ventral body rods. In the two examples of fused larvæ just described, it was found that, when skeletal parts are suppressed, such suppression is limited to the parts last to be differentiated, namely one or both dorsal rods and the aboral branches of the ventral body rods. That this suppression was permanent can readily be demonstrated. It will be recalled that all parts of a pluteus are fully formed within 72 hours, yet these fused plutei, after a period of 4 to 7 days, had not differentiated their missing rods.

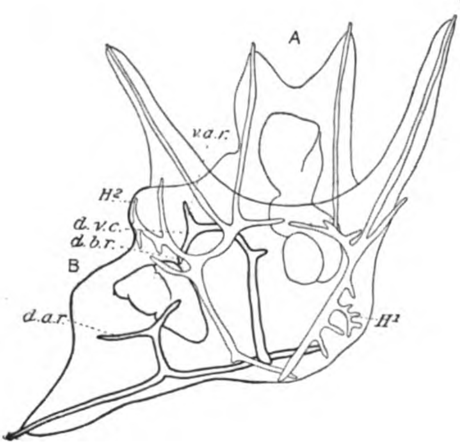

FIG. 7A.

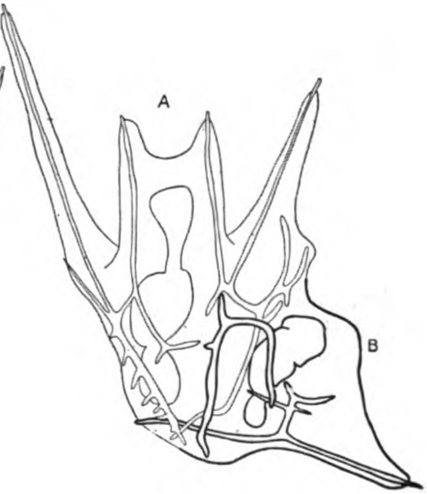

FIG. 7B.

The last fusion does not offer such clear evidence of compensatory growth as the preceeding fusion; it is probably limited to the extremely elongated dorso-ventral connectives of the B pluteus.

In the last two instances the fused plutei were equal or nearly equal, both in size and in the stage of development. In a large number of instances, however, the two plutei are unequal in both these respects and in varying degrees of inequality. For example, figures 7A and 7B are two views of the same pair of fused plutei, of which figure 7A is somewhat enlarged. In these figures the A pluteus is clearly larger and more completely differentiated than its mate; the four arms, the shape of the body, and the archenteron are completely differentiated and normal. The B pluteus appears to have but one abbreviated arm and a diminutive archenteron.

The skeletons show very clearly the unequal development of the two plutei. The A pluteus contains all the skeletal parts of the normal pluteus, and their shape and size and relative positions are essentially normal. The skeleton of the B pluteus is far from complete. It contains the two ventral body rods and two dorso-ventral connectives which are quite normal, but the two dorsal body rods, one of the ventral arm rods, and one dorsal arm rod are very much reduced in size, and there is no trace at all of the aboral branches. The compensatory growths of the skeletons in this fusion are very striking indeed. They are altogether limited to the A pluteus and confined to processes on the right ventral body rod at H^1 and on the left ventral body rod at H^2, where the outgrowth is much branched and extends into the B pluteus. The formation of these hypertrophied and supernumerary bars is explicable on the assumption that when the mesenchyme cells were prevented from developing within *its* own larva they migrated into the adjoining or A larva and there formed the additional skeleton at or near the center of active skeleton building, namely, the region of the union of ventral rods and dorso-ventral connectives.

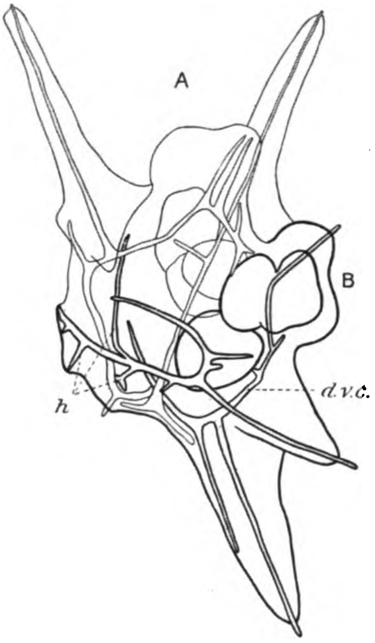

FIG. 8.

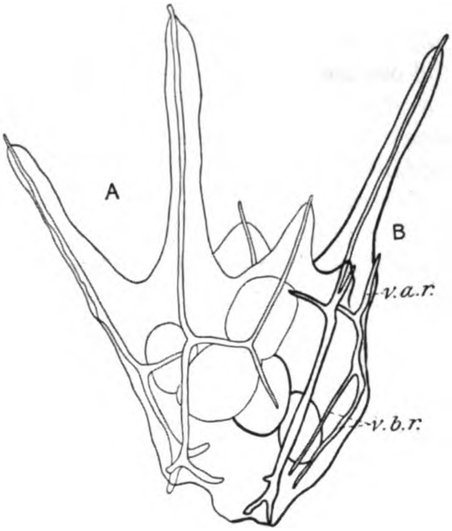

FIG. 9.

Figure 7B is a dorsal view of the same pair of fused plutei, which shows more clearly some of the points referred to.

Figure 8 represents two plutei much more completely fused into one body. The dominant or A pluteus is typical and complete; the suppressed or B pluteus is smaller and incomplete, and this inequality is shown particularly well in the skeletons. It will be observed that the B pluteus is deficient in these skeletal parts that were missing or abbreviated in the previous examples, namely, the dorsal arm rods and the aboral ventral branches. The other parts are perfectly differentiated. If the hypothesis of the migration of the mesenchyme cells from the suppressed to the other larva is correct, one would expect in this fusion correspondingly smaller compensatory growth to have taken place; this is found to be the case, namely additional processes on the ventral body rod shown at *h* and an elongated dorso-ventral connective.

A rather interesting instance of form equilibrium is seen in the A pluteus, where the left dorsal arm is absent and the right has formed an accessory arm rod and dorso-ventral connective. This fusion affords particularly strong evidence that the skeletons of the two plutei do not fuse in the ordinary acceptance of that term, for the overlapping of the parts is here very considerable.

In figure 9, the inequality of the two fused plutei is very great. The A pluteus is perfect in every detail, the B is very incomplete. In the first place, there is but one in place of four arms, and the gut is fused to the foregut of the A pluteus. The skeleton consists of two ventral body rods, only one of which possesses an aboral branch; two dorso-ventral connectives, and one large and one very small ventral arm rod. There is no trace of the dorsal arm or body rods. As in previous instances, such suppression of the skeleton is associated with compensatory growths, which, in this instance, is confined to the supernumerary ventral body rod and to a marked thickening of the other ventral body rod of the B pluteus.

The fusion shown in figures 10A (somewhat enlarged) and in 10B is particularly interesting. It resembles figure 9 in so far as the A pluteus is complete and true to type, while the B pluteus is very incomplete. The dominant pluteus has all the skeletal parts, the other (B) has but one ventral arm and an atypic archenteron. The skeleton of this pluteus consists of only three out of at least twelve parts, namely one ventral body rod, one ventral arm rod, and one dorso-ventral connective. Over three-quarters of the skeleton has not been differentiated.

The compensatory growth of the skeletal structures is to some degree commensurate with the degree of incompleteness or suppression. Close examination of figure 10 will show that the right dorso-ventral connective is not the normal single rod, but is much branched, and that from it a very long bar extends across the body of A into the body of B; it reaches almost as far as the ventral body rod of B, and might easily be mistaken for its symmetrical mate. Figure 10B affords another view of this fused larva and shows in a more striking manner some of the facts just referred to. In passing, it might be noticed that the three short rods derived from the dorso-ventral connectives of B are probably other supernumerary or compensatory outgrowths of the skeleton.

The extension of the large supernumerary (S) rod across one pluteus and into the other might be accounted for in one of two ways. Either it was formed originally as a ventral body rod in the B pluteus and secondarily united with the skeleton of the A larva at its dorso-ventral connective, or it was an hypertrophied dorso-ventral connective of A which grew beyond the limits of its own body into B. The matter can be settled conclusively only by continuous observation of the living fusion, which was not made in the case of this particular fusion. The preceding and certain of the following fusions make it altogether probable that a fusion of the two skeletons did not take place, but that the mesenchyme cells migrated from one part of the body to another, as in figures 5, 6, 8, and 9, or from one body into the other, as in figures 7 and 10. On comparing figures 7 and 10, it will be observed that the supernumerary rod in both originated in the same region, is much branched, extends from one larva into the other, differing only in the matter of length, and finally that they replace the suppressed parts of the skeleton of the weaker larva. The close similarity in these respects is significant and supports the view of a redistribution of the mesenchyme cells and the subsequent outgrowth of the hypertrophied rods into the second larva.

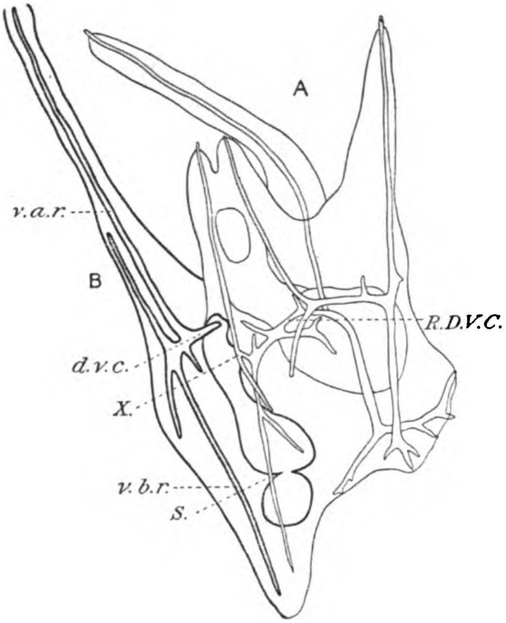

FIG. 10A.

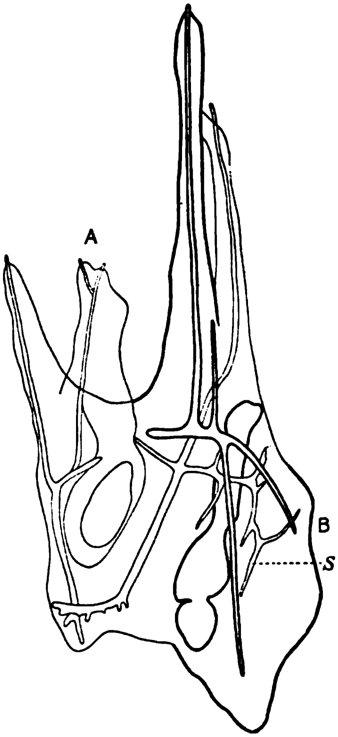

FIG. 10B.

Figure 11, which is somewhat enlarged, might be mistaken for a single larva with a supernumerary arm. On closer examination it will be found that there are clearly two larvæ fused very completely together. One of these, the dominant or A pluteus, has the characteristic four arms, perfect archenteron, etc. The other or B pluteus is represented externally by a single arm. This arm, however, contains a ventral and a dorsal arm rod united by a dorso-ventral connective, from which a characteristic ventral and a dorsal body rod extend into the common body cavity. The arm of B is really the right or left half of the B larva and if there be any doubt as to the dual character of figure 11, the diminutive archenteron in the B larva should at once set it aside.

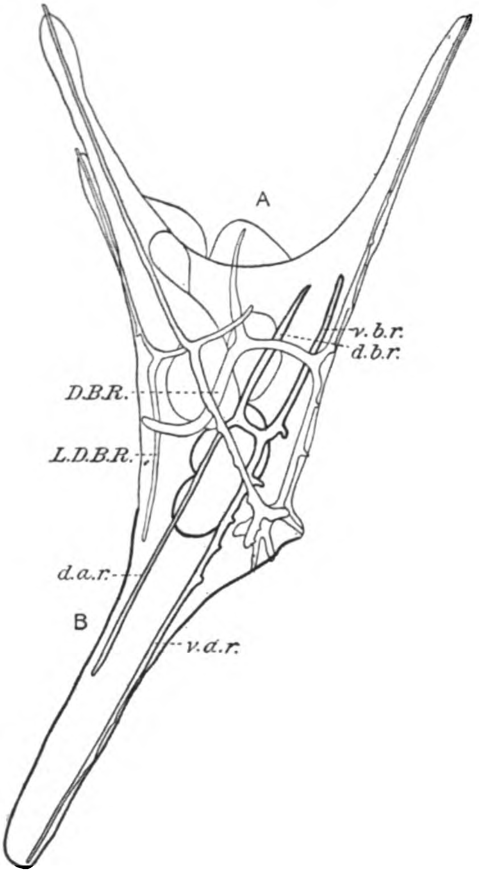

FIG. 11.

There are several instances of compensatory growths in the dominant larva. There is the hypertrophied dorso-ventral connective, and better still the dorsal body rod which is not merely considerably longer but extraordinarily thicker than in the controls, and finally an elongated left dorsal body rod.

It should again be noted that although there is considerable overlapping of adjoining skeletons there is no fusion at any point.

In the fusions of the larvæ so far described, the archentera were either independent or partially fused. In the following examples the two larvæ are more completely fused together, and there is but one archenteron, the one belonging to B having been either suppressed or fused completely in that of A.

Figure 12 is an example of a very complete fusion of two larvæ with but one archenteron. Detailed inspection shows that one larva is complete and perfect, while the other has but a short blunt arm as the external evidence of the second larva. Within this arm there is a normal and characteristic ventral body rod with a number of hypertrophied processes, a true dorso-ventral connective and an unusually thin ventral arm rod, and an accessory dorsal arm rod. In this larva the right or the left half of the larva has been differentiated, but less completely than the half larva of figure 11.

Figure 13 resembles the fusion shown in figure 12 in several essential points. Each fusion has but one archenteron, a dominant and perfect larva, and a very incomplete larva represented by a single broad arm, in which there is a ventral arm rod, a ventral body rod, and a dorso-ventral connective. In figure 13 there are two thin, straight bars—one long, the other very short, neither of which connects with either the B or the A skeletons. If it be assumed that retrogressive changes have taken place and that these have affected one or both ends of certain rods, we should expect the connections between rods to disappear, the distal ends to be shortened, and the bars themselves to be thinner than corresponding rods of control larvæ. All these results are found in figure 13 and the independent bars probably represent the former dorsal and ventral arm rods.

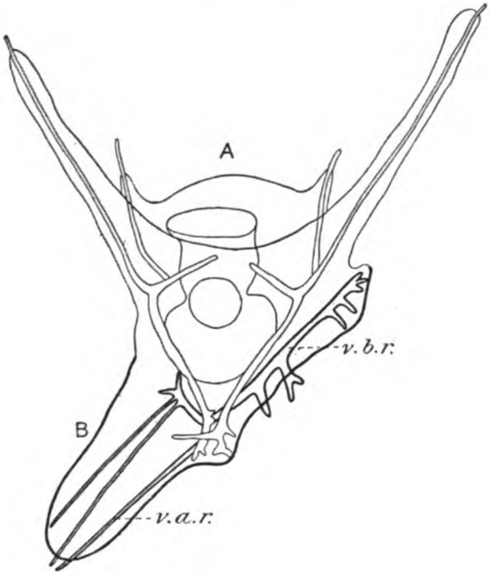

FIG. 12.

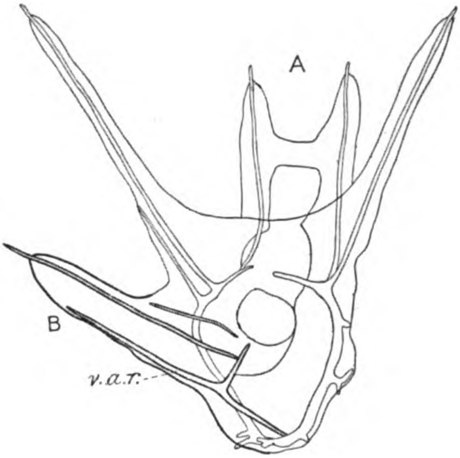

FIG. 13.

In figure 14 form equilibrium is more closely established. Larva B is reduced to a small, very blunt protuberance on the left side of the figure. It consists internally of a ventral body rod and its aboral branch, a very much dwarfed ventral arm rod, a dorso-ventral connective, a dorsal body rod, a supernumerary rod at S and a mere rudiment of the dorsal arm rod. In other words only *one half of one side of the skeleton has been formed* in this larva.

The gradual decrease in size and contents of the B larva, seen in figures 11 to 14 inclusive, reaches an extreme state in figure 15; and if it were not for the intermediate stages already described, figure 15 might easily be overlooked or mistaken for a single larva.

In figure 15 the A larva contains all the parts in their proper relative positions typical of the control larva. The gut is somewhat enlarged, indicating the incomplete fusion of the two archentera. The skeleton is single and perfect and to describe it is to describe the control larva. The B larva is represented only by a short, blunt arm containing a *single straight rod,* probably a ventral arm rod, which passes the ventral body rod of the A larva and ends blindly in the common body cavity. This single rod is less than one-twelfth of the complete skeleton whose parts have either been suppressed or disintegrated after their formation. If either or both of these processes were to continue beyond the stage shown in this figure the blunt arm would disappear with its contained single bar and all trace of the B larva would be gone. It would then be absolutely impossible to distinguish such a larva from the non-fused larvæ in the rest of the culture.

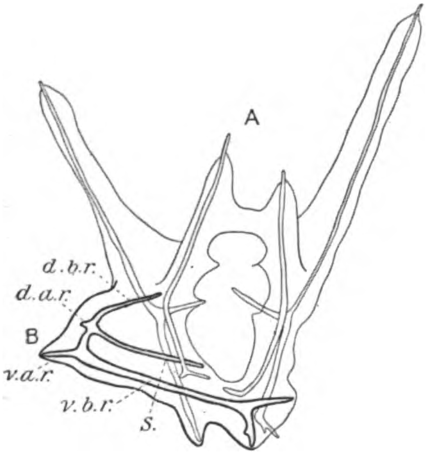

FIG. 14.

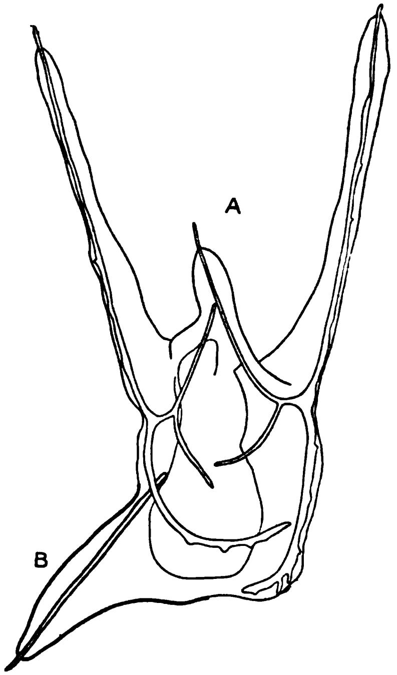

FIG. 15.

DISCUSSION.

In a previous publication I have shown that two or more blastulæ or gastrulæ derived from separately fertilized eggs, when united, tended to be remolded into a single gastrula somewhat larger than the controls. In this paper I have shown that this process of form regulation continued through the larval period, during which the tissues were completely differentiated. Such fusing larva tended to be remolded into a single one of normal size, but this tendency is conditioned by certain factors, of which the following are the most important:

(1) *Area of the agglutinated surfaces of the two blastulæ:* When the developing blastulæ are merely agglutinated by an inconsiderable area of their surfaces, each develops into a gastrula and a pluteus—complete, perfect,

and independent of the other except for the common surface of attachment. There is no reciprocal influence of one upon the other. When, however, the agglutinated surfaces extend over a large area the blastulæ tend to fuse, to have a common blastocœle, and the gastrulæ tend to approximate the form of a single embryo. It can not be stated definitely which is cause and which effect, whether greater common surface makes for more complete fusion or whether the internal forces making for fusion also affect the surfaces of the agglutinated embryos.

(2) *Mass of agglutinated or fused embryos:* When more than four or five eggs are agglutinated the disintegration of one or more of them at an early stage in their development brings about the disintegration of the entire cluster before they can fuse. When three blastulæ are agglutinated they may develop into three perfect and practically independent larvæ, or they may fuse together, under which circumstance the reciprocal influences are exceedingly complex and the resulting larva is quite atypic. For this reason I have omitted all consideration of such complex fusions and limited myself to fusions of two eggs, so that the regulative changes may be observed with the least number of disturbing factors.

(3) *Differential rate of development:* When two gastrula are fused and each has developed at an equal rate, twin larvæ like that shown in figures 5 and 6 result, in which case each larva is practically complete and equal in size, and has little or no influence upon the other. When two gastrula have not developed equally the conditions are then most favorable for form regulation.

At least two factors are involved in form regulation, namely, the suppression of structures and the disintegration of those already differentiated.

I have elsewhere shown, from the study of living fusions, that the archenteron of one of the fused gastrulæ may be entirely inhibited or may never develop beyond an early stage of its differentiation. Driesch and de Haan have also observed aborted or dwarfed archentera. I have shown in this paper that certain parts of the skeleton also may never be developed, that such suppressed parts are nearly always the last to be differentiated and confined to but one of the fused larvæ.

It has been urged by de Haan that the suppressed larva is a sick larva, that there is no regulation but that a union of a healthy and an unhealthy larva occurs. Under such circumstances we should expect the sick larva to be atypic and some relation between diminutive size and degree of irregularity in development. But the fused larvæ are quite perfect and often of normal size. It might be urged that only very sick larvæ would be atypic and would not complete their development. But how can one account for the lack of development of the right or the left sides of the skeleton on such an assumption? It would also be exceedingly difficult to account for the absence of the oral half of one side (fig. 14), particularly in the face of the fact that the skeletal parts that are differential *are characteristic and normal* and show no trace of irregularity or sickness.

Since the suppression of skeleton, arms, and archentera, etc., do not occur in control larvæ, it seems reasonable to suppose that the more rapidly developing larva influences the other in such a manner that the mesenchyme cells of the latter are prevented from completing the skeleton, and the degree of suppression seems to be related to the differential rate of development, the greater the difference the greater the suppression of the parts of the slower developing larva.

The second factor making for form equilibrium is the loss of parts that have already been differentiated. I have shown from the study of living gastrulæ that the archenteron in the slower developing gastrula in a fusion may be reduced in size and completely disappear. The skeletons may also actively degenerate in one of the fused larvæ. This degeneration is evidenced in the reduction in thickness of various rods, in the disintegration of one or both ends of rods, or both of these processes. As a result of the incomplete development of the skeleton and the subsequent disintegration of parts that have been formed the skeleton may ultimately consist of but a single or a few independent rods. And since the size of the body is so dependent upon the size of the skeleton the suppressed larva may decrease in size until it almost disappears, as shown in figure 15, and I believe may completely disappear.

What happens to the tissues of the degenerating larva? Are they absorbed as food by the dominant larva as nurse cells supply egg cells with nutriment; or are they disintegrated after the manner of certain grafts without any influence upon the host; or are they transferred and reconstructed within the body of the dominant larva? There is unmistakable evidence that two of these processes ordinarily take place, but in unequal degree and affecting different structures of the organism.

The transfer and rebuilding of the materials of one larva into the other are limited to the last differentiated tissues, namely, the skeleton. It was shown that when two fused larvæ developed unequally the dominant pluteus may prevent the completion or the development of parts of the skeleton; that under these circumstances the unused mesenchyme cells may migrate to other regions of the body or *into the body of the dominant larva* and there give rise to additional skeletal material, either by the thickening or elongation of the rods or by the growth of supernumerary processes and rods. The extent of such hypertrophied or supernumerary growths was commensurate with the suppression of skeletal parts in the other regions.

De Haan seems to have shown that two eggs may be completely fused into one somewhat larger than the control. Driesch, Goldfarb, and de Haan have demonstrated that blastulæ may be completely fused together into a single larger blastula. The evidence is not so conclusive but seems to point to a complete fusion of gastrulæ and the enlargement of the gut as well as the body.

Beyond this stage in the differentiation of the tissues a complete fusion of the organs of the two larvæ does not take place, at least in *Toxopneustes* *variegatus.* The skeleton under no circumstance fuses in the two individuals and there is no evidence of an enlargement of the larva to form the "einheits pluteus" or completely fused larva of Driesch. There is, on the contrary, definite evidence that disintegration of skeletal materials takes place in the suppressed larva *without any corresponding enlargement of the skeleton or the body of the dominant larva.* The dominant larva in figures 12, 13, 14, and 15 are no larger than in figures 9, 10B, 5, 6, 7B, and 8—all drawn to the same scale—or figures 11, 10B, and 7A, drawn to greater magnification. In the second place the dominant larva in these fusions are no larger than the corresponding control larva, and the variation in size is approximately the same in both groups. There are no giant larva in the sense employed by Driesch and Morgan and other investigators. There are completely fused larva as there are completely fused blastulæ and gastrulæ.

The facts clearly show that in the blastula, gastrula, and larval stages the less or earlier differentiated tissues (such as ectoderm and entoderm) after their union to form the giant body and giant gut diminish in size until a normal single body and gut are approximated. This diminution must occur either as a consequence of the degeneration of certain cells or the diminution in size of the cells. The latter can readily be shown not to be the case.

It can also be shown that the degenerating substances in one larva do not serve as food or stimulant of the other, for the dominant larva are no larger nor do they develop any faster than the controls. In fact they are considerably slower than the controls.

The increased growth occurs not in any increase of the larval body or of the contained organs, but in the hypertrophied and supernumerary processes and bars whose origin and development have been described.

SUMMARY.

A new method was discovered by which large numbers of eggs of *Toxopneustes variegatus* could be fused together, during the blastula and gastrula stages. The method consists essentially in placing the fertilized eggs in a solution composed of one part of sea-water to three parts of either an isotonic or a slightly hypotonic NaCl solution.

This solution has two effects upon the eggs, namely, it increases the volume of the egg and thereby bursts the fertilization membrane, and it gelatinizes the surface of the egg. When any such eggs are in contact they become agglutinated and may remain agglutinated and produce twin larvæ or fuse together during the blastula or gastrula stages.

The conditions that favored the fusion process were:

(1) The number of agglutinated eggs: More than two eggs rarely gave rise to a fused and characteristic larvæ. When two eggs were agglutinated they either developed into twin larvæ or fused more or less completely together.

(2) Time of fusion: The earlier in development that fusion occurred the more completely did the parts tend to fuse together and vice versa.

(3) Area of common surface: The greater the surface common to both eggs or blastulæ, the more completely and the earlier did they tend to fuse, and vice versa.

(4) Differential rate of development: When the two embryos developed equally fast, each was essentially perfect, though fused to the other. When one developed less rapidly than the other, reciprocal changes took place, as a result of which the larvæ tended toward complete fusion and form equilibrium.

Form equilibrium is determined by at least these three factors, namely:

(1) Differential rate in the development of the two eggs.

(2) An inhibition in the development of certain structures. The skeleton in the more slowly developing larva was rarely complete, lacking those portions of the skeleton that were last differentiated. Such portions may include one-half, three-quarters, or more of the skeleton. The parts that do appear are perfectly normal.

(3) A disintegration of certain structures. Skeletal parts of the larva after their complete differentiation may break down and completely disappear. Such decrease in the skeleton was accompanied by a decrease in the corresponding parts of the body of the larva.

Such regulative changes are nearly always limited to the more slowly developing larva. They result in the complete atrophy of this larva without any reciprocal or compensatory effect upon the shape, size, or parts of the more rapidly developing or dominant larva, except for the skeleton.

The ectoderm and the entoderm of one larva may fuse with that of the other, and may subsequently be absorbed or be disintegrated without any visible effect upon the other larva. The skeleton never fused. When it was suppressed in one larva, some or all of the unused mesenchyme cells migrated to other regions of the same larva, or into the adjoining larva, and there gave rise to hypertrophied or supernumerary parts of the skeleton, and in certain instances replaced in part the missing skeleton of the suppressed larva.

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VII.

EXPERIMENTS ON THE PERMEABILITY OF CELLS.

BY J. F. MCCLENDON,
Instructor in Physiology, University of Minnesota Medical School.

Three text-figures.

EXPERIMENTS ON THE PERMEABILITY OF CELLS.

By J. F. McClendon.

One of the most important steps in the analysis of life was the discovery of oxygen. Ever since that time it has been known that animals absorb free oxygen and give it out in a combined form.

The discovery of enzymes has given a clue to the rapidity of oxidation within cells; but enzymes are always present in living substance, and yet oxidation may vary from almost zero in certain eggs and seeds during rest to a high rate during activity. We know that oxidation within muscle is greatly increased during contraction, yet hardly anyone would suppose this to be due to the sudden increase of oxidases. Loeb, however, did use this explanation for the increased oxidation of the egg on fertilization, by suggesting that the sperm carries an oxidase into the egg. Certainly the sperm contains an oxidase, as does every other cell, but the sperm is minute in comparison with the egg, and no one has found any indication of an unusual amount of oxidase in the sperm. Furthermore, the egg may be made to divide by artificial means without the introduction of any enzyme.

An organism may be caused to absorb more oxygen by any one of a number of different stimuli. But what is stimulation? What is the first change induced in the organism or excised organ by all stimuli?

Pfeffer showed that stimulation of sensitive plants causes a change in permeability of certain cells (of the pulvinus). This change in permeability causes a change in turgor (internal osmotic pressure) and movement results. The question arises: what relation does this bear to oxidation? According to Ralph Lillie oxidation is suppressed in the cell by the accumulation of waste products to which the resting cell is impermeable. Stimulation increases the permeability of the cell to them and consequently oxidation is increased. Lillie maintained that on fertilization the egg became more permeable, and linked all subsequent phenomena with this change. Not finding any other explanation of the stimulating action of the sperm, I attempted an exact study of the permeability of the egg.

EXPERIMENTAL.

Three methods of procedure were followed: (1) the use of cell masses as partitions (on eggs of *Lytechinus*); (2) the use of quantities of eggs suspended in a liquid medium (on eggs of *Fundulus*); (3) experiments on individual eggs (of *Arbacia*).

The first of these methods was tried at the Tortugas laboratory on the eggs of the sea-urchin *Lytechinus* (*Toxopneustes*) *variegatus*. Advantage was taken of the fact that the electrolytes of the eggs and the medium dissociate into ions which bear electric charges, and therefore their movement through the eggs could be detected, with a high degree of accuracy, by the electric conductivity method of Kohlrausch.[1]

Several bushels of sea-urchins were collected each day and the eggs of the ripe females placed in large dishes of sea-water. The mucus egg membranes (jelly, zona pellucida) were washed off and the eggs were introduced into a conductivity vessel made especially for the purpose (fig. 1). The conductivity vessel was placed in the centrifuge and the eggs were precipitated until they were closely packed together. By microscopic examination it was found that only a trace of sea-water was left in the spaces between the eggs (fig. 2).

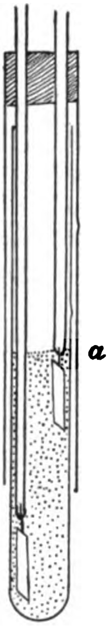

FIG. 1.

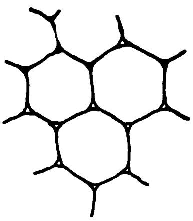

FIG. 2.

By raising the electrodes above the level of the eggs they came to lie in the supernatant sea-water that had been pressed out from between the eggs. In this way the conductivity of sea-water and egg-mass could be measured separately within two minutes. The conductivity of the egg-mass, when moderately centrifuged, was about one-twentieth of that of the sea-water, indicating that the conductivity of the egg itself must be almost nil.

The conductivity of the egg-mass was greatly affected by the degree of packing. However, the vessel was so long and narrow that the packing of the eggs could be recorded accurately by marking their upper limit on the side of the vessel (fig. 1, *a*). The eggs could then be removed from the conductivity vessel, replaced, and centrifuged to the same level, when the conductivity was found to be the same. In this way I was able to measure the conductivity before and after fertilization and before and after artificial stimulation causing cleavage. It was found that the conductivity increased about one-fourth when the developmental processes began. The sources of error and methods of controlling them are discussed in another paper.[2]

One point, however, the elimination of the fertilization membrane, needs further consideration. Soon after the egg is fertilized, a fine line appears close around it and gradually expands until it forms a circle, widely separated from the egg. Almost every one (except Kite) supposes this to represent a spherical membrane, the "fertilization membrane." Before fertilization the egg is covered by a thick transparent jelly or mucus layer, called the chorion or zona pellucida by some authors. I stained this jelly with basic dyes (neutral red and methylene blue). These dyes coagulate the jelly, causing it to become membranous.

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[1] Ostwald-Luther. Messungen.

[2] McClendon. Amer. Jour. Physiol., vol. 27, p. 240.

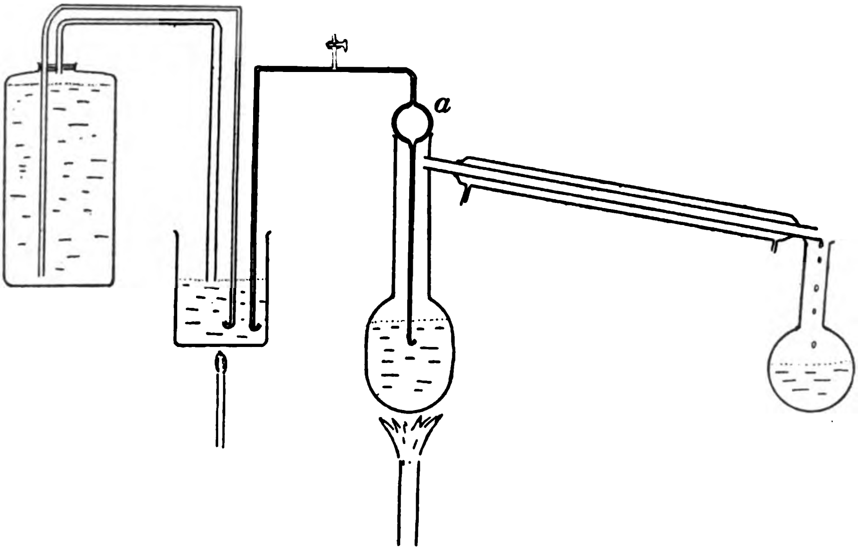

FIG. 3.

In these experiments all toxic substances were eliminated from the water by redistilling it in the apparatus shown in figure 3. The stock bottle (to the left) is filled with distilled water, which siphons over into the beaker and keeps the latter filled up to the mouth of the wide tube. A small flame under the beaker boils the water and drives out all dissolved gases (since the water contains no salts to hold back CO_2, and no non-volatile substances to liberate ammonia). The boiling water from the beaker siphons over into the Kjeldahl flask over a large flame. If the siphon be drawn out into a fairly small opening the Kjeldahl flask may be set lower than the beaker, as in the figure; but this has the disadvantage that when the still is cooled the Kjeldahl flask fills up to the neck and makes it inconvenient to start again. The siphon has a bulb blown on it at *a*, to stop the mouth of the flask. It is not necessary that this stopper be ground in, provided it fits fairly snug, as water condenses on the stopper and seals the crack. A hole is blown in the side of the neck of the flask to admit the condensing tube. This hole may be reamed out with the end of the tube itself, while the glass is hot, to insure a fairly close fit. The condensing tube is of fused quartz and the only water that collects in the receiving bottle is condensed on the quartz. The cold water dissolves glass very slowly, so that it is permissible to use a glass receiving bottle if the water is distilled fresh each day. Hard glass (Jena or Bohemian) dissolves more slowly but contains heavy metals which are toxic even in the minute quantities that dissolve away in the cold distilled water, even though the glass be previously steamed out. Soft glass liberates traces of sodium, calcium, and potassium, which render the water less toxic, but which, if present in sufficient quantity, may be a source of error in the experiment.

In a previous paper[1] I concluded that the fertilization membrane is the result of a mutual coagulation and precipitation between the jelly and another transparent colloid filling the space between it and the egg. I found that if the jelly be removed from the egg no fertilization membrane can be formed. Elder[2] subsequently made the same observation and came to the same conclusion. No matter what is the nature of the membrane, the fact that it can be suppressed is of importance in my technique, since the presence of the membrane might be considered as a source of error.

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[1] McClendon. Science, vol. 33, p. 387, Mar. 10, 1911. [2] Arch. f. Entwicklungmech., vol. 33, p. 143, 1912.

The increased conductivity of the eggs indicates increased permeability to electrically charged particles, since electrons can not pass in the free state through solutions without very soon attaching themselves to atoms. The particles concerned must be ions, since colloidal particles move slowly and carry little electricity per unit mass, and could hardly cause the great increase in conductivity observed.

This increase in permeability was confirmed by plasmolytic experiments on the eggs of *Arbacia* at Woods Hole.

What ions are concerned in the increase in permeability has not been determined. Experiments which I made on the *Fundulus* egg are suggestive.[1] *Fundulus* eggs (which normally develop in sea-water), when developing in distilled water, do not give up chlorides to the water. If placed in sulphates or nitrates of sea-water metals, they still do not give up chlorides. If placed in distilled water they do not give up magnesium (or calcium). But if placed in sodium salts they lose magnesium (and probably calcium and potassium), and chlorine, because the pure sodium increases the permeability.

A critical review of the literature on permeability is given in a previous paper[2] but I shall here refer to some of it briefly. In the summer of 1910 R. Lillie, Lyon and Shackell, E. N. Harvey, and the author, all working on the sea-urchin's egg, had come to the conclusion that its permeability increased on fertilization. Very shortly there appeared a paper by Loeb[3] in which he made this statement:

> In former papers, especially in a book published a year ago, I pointed out that the process of membrane formation, or a certain alteration of the surface of the egg, is the essential cause of the development of the egg; and I pointed out, also, that this alteration of the surface might increase the permeability of the egg, especially for hydroxyl ions.

I have found one such paper,[4] but it was directed against the view that the cell is impermeable to electrolytes, and gave evidence to show that the egg is more permeable to salts than to sugar. In the book referred to[5] Chapter XIII deals largely with permeability and ends with the following conclusion (on page 118):

> Wir kommen also zu dem Schluss, dass die Hydroxyloinen des Seewassers auch in das unbefruchtete Ei diffundieren, und dass die Membranbildung des Oxydationen im Ei nicht dadurch steigert, dass sie die Durchgängigkeit desselben für Hydroxylionen erhöht, sondern dass sie die für die Entwicklung nötigen Oxidationen auf anderem Wege entfesselt oder möglich macht.

If Loeb has found evidence that the permeability of the egg increases on fertilization, or fatty acid treatment, I would be very glad to have my attention called to it, but it is difficult to find a paper without a reference to the journal in which it is published.

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[1] Amer. Jour. Physiol., vol. 29, p. 295, Appendix II.
[2] McClendon. Biol. Bull., vol. 22, p. 113. 1912.
[3] Loeb, J. Science, n. s., vol. 32, p. 412. Sept. 23, 1910.
[4] Loeb. Univ. of California Publications, Physiol., vol. 3, No. 11, p. 81. 1908.
[5] Loeb. Die Chemische Entwicklungserregung des tierischen Eies. Berlin. 1909.

The degree of permeability is of importance in development. R. Lillie maintains that too great an increase in permeability causes cytolysis of the egg. Gray[1] attempts to explain the abnormality of Echinoderm hybrids by assuming that the foreign sperm produces the wrong degree of permeability and this leads to some of the chromatin becoming pathological and being thrown out of the spindle.

The changes in permeability are probably due to changes in the aggregation state of colloids: either proteids, lipoids, or proteid-lipoid combinations or associations. Many investigators have looked for such changes with the microscope. That some change takes place at the surface of the egg on fertilization has long been known, but opinions differ as to its exact nature. Spherical concentric surfaces, when viewed tangentially with the microscope, cause optical illusions, and the real structure can not be exactly determined without knowledge of the refractive indices of the substances composing it. Kite, however, claims that a thin layer may be observed on the surface of the unfertilized egg and, on fertilization, this layer absorbs water and swells. He claims to have stained it differentially.[2] I do not understand, however, his observations on unstained eggs. He says that this layer which he calls the vitelline membrane becomes more distinct (of different refractive index) from the sea-water on swelling, whereas it is well known that all hydrophile colloids, on swelling, become less distinct and more nearly of the same refractive index as the medium.

The swelling of this layer might cause an increase in permeability, since the swelling of all hydrophile colloids increases their permeability to water-soluble substances. Lillie, however, believes that a reversible increase in permeability takes place in the superficial layer of the cytoplasm (plasma membrane) and J. Gray who has confirmed my determination of the increase in permeability of the egg finds that impermeability returns in 15 minutes, whereas Kite's vitelline membrane remains swollen. I consider Kite's vitelline membrane a new name for a colloid postulated by Loeb.

Mitchell and the author[3] studied experimentally the role of hydroxyl ions in the increase in oxidation of sea-urchin's eggs, which follows fertilization or artificial stimulation. We concluded that the increased permeability allows the hydroxyl ions of the sea-water to penetrate the egg. This increase in hydroxyl ions within the egg might increase oxidation.

CONCLUSIONS.

The permeability of the egg to ions and perhaps some other substances increases on fertilization. The unfertilized egg is perhaps in a dormant condition and the increase in permeability probably allows a rapid interchange with the surrounding medium necessary for activity (development).

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[1] Gray, J. Proceedings of the Cambridge Philosophical Society, vol. 17, p. 1, 1913.
[2] Kite. Science, n. s., vol. 36, p. 562. 1912.
[3] Jour. Biol. Chem., vol. 10, p. 459.

Whereas this supposed significance of permeability has not been proven, the sea-urchin's egg is not an exception. Spores, eggs, seeds, and pupæ are usually inclosed in relatively impermeable membranes. A striking example is the cocoa nut. Whereas an active organism may be partly inclosed in a relatively impermeable skin, great activity is always associated with a rapid interchange with the medium, and this is possible through more permeable portions (lungs, gills, kidneys, gut).

The relation of permeability to oxidation can hardly be determined until more is known about the mechanism of animal oxidations. These seem to depend on structure since complete oxidations cease when structure is completely destroyed. Reference is made only to oxidations resulting in the formation of $CO._2$ Oxidizing enzymes such as tyrosinase, which are independent of structure, do not completely oxidize the substances acted on.

VIII.

THE RELATION BETWEEN THE RATE OF PENETRATION OF MARINE TISSUES BY ALKALI AND THE CHANGE IN FUNCTIONAL ACTIVITY INDUCED BY THE ALKALI.

By E. NEWTON HARVEY,
Instructor in Physiology, Princeton University.

One figure.

THE RELATION BETWEEN THE RATE OF PENETRATION OF MARINE TISSUES BY ALKALI AND THE CHANGE IN FUNCTIONAL ACTIVITY INDUCED BY THE ALKALI.

By E. Newton Harvey.

OBJECT AND METHOD.

The present study, made at Tortugas in the summer of 1911, is a continuation of permeability investigations undertaken at Columbia University in 1910 to 1911.[1] My aim has been twofold. First, to compare the permeability of the cells and tissues of salt-water organisms with those of fresh-water forms. Second, to determine the relation between the rate of penetration of the alkali and the appearance of structural or functional changes in the cell. Rate of penetration of the alkali may be best determined by the color change of some indicator within the cell. As in previous work, neutral red was used for the purpose. It is non-toxic, readily staining the living cell, and the color change (of red in acid or neutral to yellow in alkaline solution) is sharp and well marked.

Neutral red was first made use of as an indicator in studying permeability by Bethe.[2] Medusæ stained in the dye become a deep orange-red, which changes to bright red in HCl and to yellow in NaOH.

Warburg[3] found that sea-urchin eggs stained in neutral red were entered readily by NH_4OH but not by NaOH. My own experiments[4] gave similar results for another species of sea-urchin (*Toxopneustes variegatus*) and for *Paramœcium*, *Elodea*, and *Spirogyra* cells.

The penetration of the following alkalies was studied:

1. *Weakly Dissociated:*
   Ammonia—NH_4OH
   Methylamine—NH_3CH_3OH
   Dimethylamine—$NH_2(CH_3)_2OH$
   Trimethylamine—$NH(CH_3)_3OH$
   Ethylamine—$NH_3C_2H_5OH$
   Normal propylamine—$NH_3C_2H_5CH_2OH$
   Isopropylamine—$NH_3(CH_3)_2CHOH$
2. *Strongly Dissociated:*
   Tetraethylammonium hydroxide—$N(C_2H_5)_4OH$
   Sodium hydroxide—NaOH
   Potassium hydroxide—KOH
   Strontium hydroxide—$Sr(OH)_2$

The addition of the stronger alkalies to sea-water precipitates the Mg so that a Mg-free sea-water containing 0.625 m. (100 NaCl + 2.2 KCl + 2.5 $CaCl_2$) was used to dissolve the alkali. Such a solution is a fairly well-balanced medium for marine tissues, being only slightly inferior to van't Hoff's solution. The salt present in solution with the alkali affects also the rate of entrance of the latter, so that it is important to use always the same type of salt solution for comparative results. As stated above, Mg-free sea-water of 0.6 m. concentration was employed.

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[1] Harvey, E. N. Jour. Exp. Zool., vol. 10, p. 507. 1911.
[2] Bethe. Pfluger's Archiv, vol. 127, p. 219. 1909.
[3] Warburg, O. Zeit. f. Physiol. Chem., vol. 66, p. 305. 1910.
[4] Harvey, E. N. Loc. cit.

The magnitude of the difference between pure NaCl and a balanced medium is brought out in table 1.

TABLE 1.

Egg.	Concentration of NaOH.	Color change in Mg-free sea-water.	Color change in 0.6 m. NaCl.
		min.	min.
Toxopneustes variegatus	N/100	10 to 12	5
Palolo-worm (Eunice fucata)	N/80	12	5
Holothuria floridana (immature)	N/80	6	1

PART I. THE PERMEABILITY OF MARINE TISSUES.

PENETRATION RATE AND DEGREE OF DISSOCIATION.

Just as in the case of fresh-water organisms (*Paramœcium*, *Spirogyra*, *Elodea*), the weakly-dissociated alkalies (group 1) penetrate marine tissues almost instantly, the strongly dissociated only after an interval of 30 to 60 minutes. This statement applies to all the cells or tissues of salt-water organisms investigated, including the following:

- *Holothuria floridana*, immature eggs.
- *Holothuria floridana*, respiratory tree.
- *Hipponoë esculenta*, mature eggs.
- *Toxopneustes variegatus*, mature eggs.
- *Toxopneustes variegatus*, plutei.
- *Sabella* sp., tentacles.
- *Eunice fucata* (palolo-worm), segmenting eggs.
- *Eunice fucata* (palolo-worm), trochophores.
- *Pomatostegus stellatus*, mature eggs.
- *Cassiopea xamachana*, gonads.
- *Cassiopea xamachana*, subumbrella epithelium.
- Two unidentified species of marine algæ.

Thus, the anomalous result again appears—that it is the least dissociated, the weakest group of alkalies, which penetrate living cells most rapidly. Two of the members of this group are considerably less dissociated than the rest—ammonia and trimethylamine. We might expect them to penetrate most rapidly of all if rate of penetration bears an inverse relation to degree of dissociation. There is, however, no marked difference in penetration rate between ammonia or trimethylamine and the other amines,[5] but a well-defined difference in toxicity.

TOXICITY AND DEGREE OF DISSOCIATION.

The lower toxicity of ammonia and trimethylamine is indicated in table 2. Efficiency in preventing development of the sea-urchin's egg and cytolytic action were taken as criteria of toxicity. In addition to ammonia and the amines, tetraethylammonium hydroxide was also studied. Although a substituted ammonia, it is strongly dissociated, ranking with the inorganic alkalies, and in table 2 it may thus serve as a representative of the inorganic alkalies. The unfertilized eggs were placed in the solution, and a certain number removed to sea-water after intervals of 2 and 5 minutes and fertilized.

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[5] Ammonia and the amines enter cells so rapidly that it is impossible to detect constant differences in penetration rate.

TABLE 2.—*Effect of ammonia, amines, and tetraethylammonium hydroxide on toxopneustes eggs, stained in neutral red.*

Solution of alkali in Mg-free sea-water N/250-concentration.	Color change of red to yellow.	Effect of solution on egg after 3 hours.	Condition of eggs removed to sea-water after 2 minutes and fertilized. Examined after 3 hours.	Condition of eggs removed to sea-water after 5 minutes and fertilized. Examined after 3 hours.
NH_4OH	Instantly	Irregular fragmentation and globule formation.	Segmentation; some irregular.	Many irregular segmentations.
Methylamine	Instantly	Clear cytolysis	Irregular segmentation.	Unsegmented eggs; a few fragments.
Dimethylamine	Instantly	Clear cytolysis	Irregular segmentation.	Unsegmented eggs; a few fragments.
Trimethylamine	Instantly	Irregular fragmentation and globule formation.	Segmentation; some irregular.	Many irregular segmentations.
Ethylamine	Instantly	Clear cytolysis	Irregular segmentation.	Unsegmented eggs and fragments.
Propylamine	Instantly	Clear cytolysis	Irregular segmentation.	Unsegmented eggs and fragments.
Isopropylamine	Instantly	Clear cytolysis	Irregular segmentation.	Unsegmented eggs; some irregular segmentations.
Tetraethylammonium hydroxide.	Not yellow after 3 hours.	Many red eggs, and some fragments.	Irregular segmentation; red in color.	Irregular segmentations; red in color.

I. Control—fertilized in Mg-free sea-water—red, segmentations normal.
II. Control—unfertilized in Mg-free sea-water—red, unsegmented, normal.

It will be noticed that eggs are not cytolyzed after 3 hours immersion in N/250 NH_4OH, $NH(CH_3)_3OH$, and $N(C_2H_5)_4OH$ (column 3) and after 5 minutes immersion are still capable of fertilization (column 5); whereas the opposite is true of the remaining alkalies of table 2. $N(C_2H_5)_4OH$, most highly dissociated of all, is no more toxic than NH_4OH, least dissociated of all. The explanation of the difference must lie in the penetrating powers of the two alkalies. $N(C_2H_5)_4OH$ can not penetrate the egg-surface and is consequently less toxic than NH_4OH, which readily penetrates. Penetration is of first importance in determining toxicity. If the alkali penetrates readily, then degree of dissociation is a further factor in determining toxicity. Those weak (ammonia and trimethylamine) alkalies which are least dissociated are least toxic.

The same fact appears when other cells are tested; for if segmenting palolo eggs (*Eunice fucata*) are placed in N/250 solutions of NH_4OH and the amines for two minutes, and then returned to sea-water, only those eggs develop into swimming larvæ which have been immersed in trimethylamine and ammonia. Eggs exposed to the remaining amines are for the most part killed; only a few larvæ develop.

GENERAL CONSIDERATIONS.

On being returned to sea-water the palolo eggs which have been turned yellow by immersion in the weak alkalies again become red in color. The return of color is much more rapid in ammonia and trimethylamine than in the remaining amines. The explanation of this phenomenon lies in the lesser degree of dissociation of the former substances. Neutral red occurs in the egg in small granules, a combination between the neutral red base (BOH) and some organic substance having acid properties (HA), forming a salt (BA).

$$BOH + HA = BA + H_2O$$

This red salt BA is broken up by NH_4OH (or any other alkali), neutral red (BOH) is freed, and a compound NH_4A is formed. The weaker the alkali the greater will be the hydrolytic splitting which its salt, NH_4A, will undergo.

$$NH_4A + H_2O = NH_4OH + HA$$

The NH_4OH will diffuse away more rapidly than a stronger base, such as methylamine, and recombination of HA and BOH will consequently occur more rapidly with the reappearance of the red color.

The results with marine organisms agree in general with those obtained on fresh-water forms. One point of difference is worthy of mention. Ammonia and trimethylamine were found to be less toxic than the remaining amines for *Elodea* cells, just as in the case of marine forms; but while ammonia was less toxic, trimethylamine was ranked with the remaining amines, not with ammonia, for *Paramœcium.* I am unable to explain this difference. It is possible that my result with trimethylamine and *Paramœcium* may be due to some unforeseen experimental error. In other respects the results agree entirely.

The effect of alkalies on living tissues in general may be represented as follows:

Strongly dissociated inorganic alkalies, including tetraethylammonium hydrate.	More strongly dissociated of the weak alkalies. Amines (except trimethylamine).	Less strongly dissociated weak alkalies. Ammonia and trimethylamine.
Penetration very slow. Least toxic.	Penetration very rapid. Most toxic.	Penetration very rapid. Less toxic.

PART II. PERMEABILITY AND FUNCTIONAL CHANGE.

THEORETICAL.

Two quite opposite points of view have been taken by physiologists in regard to the relative importance of the permeability of cells. Some regard the cell-surface as of no consequence in regulating cell processes, being freely permeable at all times to crystalloidal substances in the medium or in the cell. Others see in the surface an important regulator of chemical reactions in the cell and a barrier of a high degree of impermeability, retaining cell constituents as a test-tube retains the substances reacting within its walls. Theoretically, no more simple method of regulating the velocity of chemical reactions could be found than by means of a membrane whose permeability to the reaction product might vary. Actually, there is a large mass of evidence indicating that the cell-surface is a highly impermeable membrane for substances normally occurring in or used by cells. The evidence has been advanced in numerous papers by Lillie[6] and Hoeber[7] and need not be discussed here.

Crucial evidence has only recently been put forward that the cell-surface (particularly its permeability) plays an important part in cell activities—that it may vary at different periods of activity and in the presence of definite substances.

The evidence may be classified under three heads:

1. Measurements of permeability changes induced by narcotics, anæsthetics, and salts or other substances which affect cell activities.
2. Measurements of permeability during different states of functional activity.
3. Determinations of the rate of penetration of a substance and rate of change in functional activity induced by the substance.

PERMEABILITY CHANGE THROUGH CHEMICALS.

Of particular interest here is the experiment of Osterhaut,[8] in which it is shown most clearly by the plasmolytic method that the plasma membrane possesses greatest impermeability to salts in a balanced medium or in the medium which most nearly approaches that of the normal. Pure NaCl or pure $CaCl_2$ both enter *Spirogyra* cells or marine algæ slowly, the latter more slowly than the former; but if the two are mixed in the proportions of a balanced solution, they mutually retard each other's entrance. The NaCl, alone in solution, must increase the permeability of the plasma membrane to such an extent that its entrance is greatly facilitated.

Osterhaut[9] has recently confirmed his results, mentioned above, by the electrical conductivity method of measuring permeability. Disks cut from the kelp, *Laminaria*, were placed face to face so as to form a cylinder several centimeters long, and its conductivity was measured. In NaCl the conductivity of the cylinder was found to increase regularly (as compared with its value in sea-water), while in $CaCl_2$ or lanthanum chloride the conductivity decreased. Under the conditions of the experiment, increase or decrease in conductivity denotes a corresponding increase or decrease in permeability. The changes in permeability induced by NaCl, $CaCl_2$, or $LaCl_3$ are readily reversible within certain limits on returning the tissue to sea-water.

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[6] Lillie, R. S. Amer. Jour. Physiol., vol. 24, p. 14. 1909; vol. 28, p. 197. 1911. Also Biol. Bull., 27, 192. 1909.

[7] Hoeber, R. Physikalische Chemie der Zelle und Gewebe., 3d Aufl. 1911.

[8] Osterhaut, W. J. V. Science, n. s., vol. 34, p. 187. 1911.

[9] Osterhaut, W. J. V. Science, n. s., vol. 35, p. 112. 1912.

My own experiments, cited above, indicate that NaOH may enter many kinds of eggs much more readily when dissolved in 0.6 m. NaCl than in 0.625 m. Mg-free sea-water. The pure NaCl evidently decreases the resistance[10] of the egg-surface for NaOH.

The surface of *Elodea* and *Spirogyra* cells is likewise affected by minute traces of substances in solution. For instance, N/40 NaOH dissolved in tap-water entered *Spirogyra* in about 40 minutes; dissolved in double distilled water, condensed in soft glass,[11] in about 10 minutes; and dissolved in N/10 NaCl, instantly. Again the resistance of the surface is greatest in the medium most nearly normal. Addition of $CaCl_2$ to the NaCl prevents so rapid penetration of NaOH.[12]

Anæsthetics give similar results with *Elodea* cells. Chloroform added to N/40 NaOH to 1/6 saturation increases the penetration rate from 90 to 13 minutes. The protoplasmic rotation characteristic of the *Elodea* cells ceases in 1/6 saturated chloroform, but the effects of the chloroform are completely reversible, for, on removing the leaves again to pure water, rotation promptly begins. Alcohol and ether act in a similar manner.

That chloroform increases permeability in 1/6 saturated solution may be verified by the plasmolytic method. Such "reversibly" chloroformed cells are plasmolyzed by urea much less readily than normal cells, a result indicating that the urea enters more rapidly.[13]

On the other hand, Lillie[14] cites evidence to show that very small concentrations of anæsthetics decrease permeability.

PERMEABILITY AND FUNCTIONAL ACTIVITY.

Lillie[15] has advanced evidence showing that stimulation of muscle-tissue is accompanied by an increase in permeability of the tissue. Those sodium salts which are most effective in stimulation are most effective in causing the loss of pigment from the tissues of *Arenicola* larvæ. Loss of pigment indicates increased permeability to pigment. The same is true as regards artificial parthenogenesis. These salts most effective in stimulating development cause most rapid loss of pigment from the eggs (*Arbacia*);[16] they are also most toxic; in them death of the egg ensues most rapidly. It has long been known that the condition of death is one characterized by marked increase in permeability. The change on stimulation is in the direction of that accompanying death.

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[10] The word resistance is used instead of permeability to indicate that the cell is normally impermeable to NaOH which only enters after affecting the cell-surface.

[11] Such water is perfectly harmless for both *Spirogyra* and *Paramæcium*, a good test of the purity of a water.

[12] Harvey, E. N. Loc. cit., p. 540.

[13] Harvey, E. N. Loc. cit., p. 539.

[14] Lillie, R. S. Amer. Jour. Physiol., vol. 29, p. 372, 1912; vol. 30, p. 1, 1912.

[15] Lillie, R. S. Amer. Jour. Physiol., vol. 24, p. 14. 1909.

[16] Lillie, R. S. Amer. Jour. Physiol., vol. 26, p. 106. 1910.

Further evidence that the egg increases in permeability immediately after fertilization is furnished by McClendon.[17] He found that the electrical conductivity of the sea-urchin egg is increased after fertilization, thus showing that its permeability is increased.

A similar result may be obtained by the plasmolytic method.[17] Fertilized eggs are plasmolyzed more readily than unfertilized by a molecular solution of cane sugar. This is due to the fact that the salts diffuse out of fertilized eggs more rapidly, the osmotic pressure within falls more rapidly, and plasmolysis occurs more readily.

Yet another fact indicates that the surface of fertilized eggs has undergone a marked change. They are entered more readily by NaOH.[18] In this particular case we may say that the resistance to the entrance of alkali has decreased, using the word resistance, as before, to indicate that the NaOH enters only after modifying the normal impermeability of the surface.

Tröndle,[19] and also Lepeschkin,[20] using the plasmolytic method, have found that the permeability of plant cells for salts and sugar increases in the light. The increased permeability has an adaptive significance in that the sugar formed by photosynthesis may be more rapidly removed and photosynthesis itself thereby favored.

PENETRATION RATE AND CHANGES IN FUNCTIONAL ACTIVITY.

Under this head are classed the cases in which the rate of penetration of the substance is reduced to zero, *i. e.*, cases in which marked functional or structural changes appear, while we have no evidence that the substance enters the cell at all. The obvious conclusion is that it produces the effects observed by acting on or combining with the cell-surface itself, and affords excellent proof of the important rôle played by the surface in all activities.

It has long been known that muscles lose their irritability in isotonic solutions of potassium salts. Overton[21] showed that the muscle might remain in the potassium salt solution for days without change of volume, so that the potassium salt could not have entered and its effect in changing irritability must have been a surface action.

Warburg[22] showed that the oxygen consumption of the egg was greatly increased in the presence of dilute NaOH, although the NaOH did not enter; yet NH_4OH could enter readily and in concentrations containing too few OH ions to affect to any marked extent the oxygen consumption. Neutral red is therefore a more delicate test for OH ions than increased rate of oxidation. Here is unequivocal evidence that a chemical process in a cell can be affected by a substance which does not enter the cell at all.

In my earlier paper I have shown that initiation of cell division in sea-urchin eggs, stoppage of ciliary movement and cytolysis of *Paramœcium*, and stoppage of protoplasmic rotation in *Elodea* might be brought about before any appreciable amount of NaOH had entered the cells.

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[17] McClendon, J. F. Amer. Jour. Physiol., vol. 27, p. 240. 1910

[18] Harvey, E. N. Science, n. s., vol. 32, p. 565. 1910.

[19] Tröndle. Ber. d. deutsch. bot. Ges., vol. 27, p. 71, 1909; and Jahrb. f. wiss. Bot., vol. 48, p. 171, 1910.

[20] Lepeschkin. Ber. d. deutsch. bot. Ges., vol. 26*a*, pp. 198, 231, and 724, 1908; and Beihefte z. bot. Zentralb., vol. 24I, p. 308, 1910.

[21] Overton, E. Pfluger's Arch., vol. 92, p. 115. 1902.

[22] Warburg. Loc. cit.

It is the purpose of the following paragraphs to indicate how far other functions may be affected by NaOH before it penetrates the cell. It may be stated in advance that every cell activity thus far studied is affected, indeed abolished, before the NaOH enters, while a great many cell activities remain entirely unaffected even after NH_4OH has penetrated and turned the neutral red to yellow.

Evidence showing that the indicator method is an adequate one for detecting the penetration of both strong and weak alkalies has been presented in a previous paper (Amer. Jour. Physiology, vol. 31, 1913).

EXPERIMENTAL.

MUSCLE AND NERVE TISSUE.

CASSIOPEA XAMACHANA.

The only experiments on the permeability of the cells of medusæ of which I am aware are those of Bethe, cited above.

The method of experimentation with *Cassiopea*, a scyphomedusa well adapted for physiological investigation, was the same as that previously used in studying the effect of temperature on the muscular contraction and nerve conduction.[23] The method was originally described by Mayer.[24] A long strip of tissue cut from the disk of the jelly-fish is laid across three dishes (A, B, and C, fig. 1). Dishes A and C are filled with sea-water. Dish B is filled with the alkali solution to be tested. Stimuli are constantly arising in the sense-organs in dish A and pass along the nerve-network of the strip of tissue toward C, stimulating the muscles as they go. When the nerves are so affected by the alkali in B that they no longer conduct a stimulus, the muscle in C will no longer contract. In the case of some alkalies, acids, and most other substances, the *muscles* in B are affected before the nerves. The time required to stop contraction can be determined by mere inspection, since the muscles are being constantly stimulated by nervous impulses carried from the sense-organs in A.

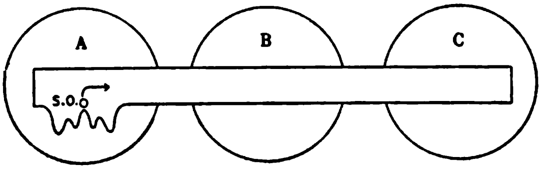

FIG. 1.

In the case of certain of the alkalies, the *nerves* in B are affected before the muscles. We may then determine by direct stimulation of the muscles in B with the electric current when they have been so far affected as to cease contracting.

Entrance of alkali is of course detected by the color change of neutral red.

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[23] Harvey, E. N. Carnegie Institution of Washington, Pub. No. 132, p. 27. 1911.
[24] Mayer, A. G. Carnegie Institution of Washington, Pub. No. 102, p. 128. 1908.

The subumbrella surface of *Cassiopea* is covered by a thin layer of epithelium and muscle and nerve tissues. The epithelium is outermost, nerve-network next, then come the muscle-fibers, and finally, embedded in the jelly, are clumps of symbiotic algal cells scattered in lines running parallel with the muscle-fibers.

Microscopic examination of the *Cassiopea* strips used in the following experiments reveals a layer of red-stained granules near the surface and on focusing downward *lines* of red-stained granules are observed following the muscle-fibers and appearing to be within them. Transverse sections of the same strips showed, however, that very little more than the outer third of the epithelio-muscle layer contained cells stained in neutral red. The dye is chiefly taken up by globules and granules in epithelial mucus-cells. These granules are practically all cast off in the mucus formed by exposing *Cassiopea* to chloroform-saturated sea-water or other injurious solutions. Neither nematocysts nor algal cells stain. No definitely stained nerve-cells can be made out, although red-stained granules are present where nerve-cells should be. Only the region of the muscle-fibers away (*i. e.*, toward epithelium) from the contractile fibrils stain. It should be borne in mind, then, in interpreting the following results, that red-stained granules are not present in the immediate region occupied by the contractile fibrils, but are present in a region first reached by alkali diffusing into the tissues and in a region containing the nerve-network, although it is impossible to say whether granules within the nerve-cells are stained or not. It is probable, however, that both nerve-cells and the outer ends of the muscle-fibers contain the red indicator. The results are given in the following table:

TABLE 3.—*Relation between color-change, cessation of contraction, and cessation of conduction in Cassiopea. (Tortugas, July 9–10, 1911.)*

Alkaline solution N/250 concentration in Mg-free sea-water.	Time of exposure to solution in minutes.	Color change in minutes.	Cessation of contraction in minutes.	Cessation of conduction in minutes.	Recovery of contraction in minutes on removal to sea-water.	Recovery of conduction in minutes on removal to sea-water
Ammonia......	20	1.5	Feeble, 5; ceases, 10.....	Missing,* 10; ceases, 16.	5	2
Methylamine...	5	1	2 to 2.5.......	2............	Slight contraction in 10.	6
Dimethylamine.	5	1	Slight contraction, 4; ceases, 4.5.	2............	4...........	1
Trimethylamine.	25	2.5	Feeble, 10; ceases, 18...	Missing, 15; ceases, 20...	5...........	2
Ethylamine....	5	1	Feeble, 3; ceases, 4....	Missing, 2; ceases, 3....	6...........	6
Normal propylamine.	5	1	Feeble, 4; ceases, 4.5...	2............ 2............	5...........	5
Isopropylamine.	5	1	Feeble, 4; ceases, 5....	Missing, 3; ceases, 3.5...	1...........	1
Tetraethylammonium hydroxide.	5	No color change	Feeble, 5; ceases, 8....	Missing, 2; ceases, 4....	1...........	Missing, 2; regular, 5.
NaOH.........	10	No color change	Muscles contract when impulse passes. Very feeble, 10.	Occasional, 3; ceases, 4.....	1...........	1
KOH..........		No color change	Feeble, 8......	Ceases, 6....		
Sr(OH)$_2$.......		No color change	Feeble, 7......	Ceases, 4.5...		

* "Missing" indicates that sometimes the impulse can pass from A to C, sometimes not.

The actual times vary somewhat in different experiments, but the above is typical. The results of interest in the above table may be summarized as follows:

The weak alkalies (first seven) enter almost instantly, yet contractions and conduction do not cease till some time *after the color change occurs.*

The strong alkalies (last four) do not enter until *long after contraction and conduction have ceased.*

In ammonia and trimethylamine the muscles regularly lose their power of contraction before the nerves. In the remaining alkalies the nerves cease to function before the muscles as direct stimulation shows. The phenomenon is more marked with the inorganic hydroxides. Thus far these alkalies are the only substances known which affect the nerves first and the muscles afterwards.

Recovery of both contraction and conduction will take place if the tissue is removed to sea-water soon after stoppage of contraction or conduction occurs. Occasional exceptions to this rule have been recorded in which the nerve-tissue has been so injured in one spot that the impulse was unable to pass. The red color returns only in the tissue exposed to the *weak* alkalies.

Recovery from the effects of the *strong* alkalies never occurs if the tissue is allowed to remain in the solution long enough for the color change to take place, nor does the red color return on transfer to sea-water.

Ammonia and trimethylamine are less toxic than the remaining amines. The strong alkalies take an intermediate position between the two groups of weak alkalies as regards toxicity for *Cassiopea* muscle.

That the resistance to the entrance of neutral red is a property of the living cell may be shown by treating the *Cassiopea* strips with chloroform-saturated sea-water. The cells are killed and the red-stained granules are cast out in a slime from which the neutral red dye slowly diffuses. If such a strip is placed in N/250 NaOH, the slime is turned yellow in less than two minutes, *i. e.*, just as rapidly as in N/250 NH_4OH. To a certain extent the slime prevents free access of NaOH to the tissue. When stripped off with a camel's-hair brush, the underlying tissue, still pinkish in color, is rapidly turned yellow by the alkali. The slime forms most rapidly in the strong alkalies, much less rapidly in the stronger amines, and very slowly in NH_4OH and trimethylamine.

In the following experiments on tissues other than those of *Cassiopea* and on eggs, the effect of NaOH has always been compared with that of NH_4OH, for two reasons: (1) My general experience indicates that NH_4OH may be taken as a representative of the weak alkalies and NaOH as a representative of the strong. (2) Since NaOH always affects the cell before it enters, we might consider the visible alteration in function a more delicate test for the presence of the OH ion within the cell than the neutral red indicator. The control experiment with NH_4OH shows that this is not the case. The color change *always begins* and in many cases is complete before any alteration in function occurs. Even though NH_4OH enters readily it is too weak an alkali to affect the cell immediately.

PENNARIA TIARELLA.

The tentacles were stained in neutral red and the time for color change was compared with the time required for spontaneous movement to cease. Globules much like those occurring in *Cassiopea* take up the dye. They are present in all parts of the tentacles. It is probable (but can not be stated positively) that stained granules are present within muscle-fibers.

In NaOH of any concentration movement always ceases long *before* any red granules are turned yellow.

In N/1000 NH_4OH movement ceases in about 15 minutes. The red begins to change, but is not completely yellow at this concentration for a long time. In N/500 NH_4OH movement ceases before the color change is complete.

The above results were also obtained using the gill muscles and tentacles of an unidentified *Amphitrite*-like annelid from Tortugas, Florida.

SALPA DEMOCRATICA.

Red-stained granules undoubtedly occur within the muscle-fibers of the circular bands of this animal.

In NaOH of any concentration movement ceases *before* the color change.

In N/1000 NH_4OH contractions cease in about 15 minutes. The color change begins in 5 minutes, but is not complete even after an hour. In N/500 NH_4OH contractions cease before the color change is complete.

CILIA AND MODIFIED CILIA (CTENOPHORE SWIMMING-PLATE).

BERÖE OVATA.

Granules stained in neutral red occur in the swimming-plate cells, not in the plates themselves.

In NaOH the movement always ceases long *before* the color change occurs.

In N/500 NH_4OH, the plates cease beating at first, begin again in less than a minute, but have mostly stopped in 5, and all by 10 minutes. The red granules are only partially yellow even after 45 minutes, although the color change begins in the course of 3 to 4 minutes.

TOXOPNEUSTES LARVÆ.

Red granules occur in the ciliated cells, but not in the cilia themselves.

In NaOH the cilia always stop *before* the color change occurs.

In N/500 NH_4OH color change occurs in 1 to 2 minutes. Movement is stopped at first, begins again in about 4 minutes, continues slowly after half an hour, and has not entirely ceased even after an hour.

A similar result is obtained with palolo trochophores (*Eunice fucata*).

These experiments with marine cilia agree in every way with those obtained on *Paramœcium*.

The above results taken as a whole admit of only one interpretation. If NaOH prevents the normal functioning of many different types of cells without appreciably entering those cells, we must conclude that its effect is on the surface and that the alteration in function is connected with a change in the surface layer. Muscle contraction, nerve conduction, and the movement of cilia and modified cilia (swimming-plates of the ctenophores) must all be dependent, primarily, on changes in the surface layer of the muscle, nerve, or ciliated cells.

CYTOLYSIS OF MARINE EGGS.

Cytolysis of marine eggs is accompanied by (1) an increase in volume of the egg as a whole; (2) in many cases a solution of the yolk and pigment granules.

Two quite distinct views have been held as regards the cause of the swelling: (1) Swelling may be due to an increase in permeability of the egg surface-membrane such that the salts of sea-water can readily pass in; they therefore no longer balance the osmotic pressure of substances within the egg and the egg swells; (2) swelling may be due to a breakdown of protein, lipoid, or other substances and the development of a higher osmotic pressure or a swelling pressure within the egg. According to the first view, the action of a cytolytic substance must be chiefly on the surface; according to the second, action is on the cell contents. The relation of cytolysis to penetration of alkali is therefore of special interest as furnishing evidence in favor of one or the other theories of cytolysis. Details of typical experiments are given below.

TOXOPNEUSTES VARIEGATUS (MATURE EGG).

N/80 NaOH—Egg remains in solution absolutely unchanged for 6 minutes; diameter 5.5 units;[25] then swells suddenly to 7.0 units. Color change is coincident with swelling.

N/80 NH_4OH—Color change instantaneous. Egg remains in solution for 15 minutes unchanged; diameter 5.5 units. In the next 5 minutes the egg begins to appear coarsely granular and at the end of 2 more minutes has swollen to 7.5 units and is filled with a mass of globules. Swelling is relatively rapid.

EUNICE FUCATA (PALOLO WORM) MATURE EGG.

N/80 NaOH—Egg remains in solution unchanged for 8 minutes; diameter 19 units. Then swelling begins and continues slowly for 5 minutes; diameter 21.5 units. The color change occurs gradually during the swelling.

N/80 NH_4OH—Color change instantaneous. Egg remains unchanged in appearance and size and only becomes broken up into oil-like globules after an hour.

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[25] A unit is 19.2 microns.

HOLOTHURIA FLORIDANA (IMMATURE EGGS).

N/80 NaOH—Eggs remain unchanged in size and appearance for 6 minutes; diameter 14.2 units. The surface layer then becomes clear, the clear region extends inward, and in the course of 1.5 minutes more the whole egg is clear and swollen to 16 units. The color change occurs as the egg contents become clear.

In N/160 and N/320 NaOH the color change occurs some time before the egg clears and swells. Thus in N/160 NaOH the color change occurs in 18 minutes and cytolysis in 40 minutes.

N/80 NH_4OH—Color change is instantaneous. Eggs remain the same in appearance and size for an hour; diameter 14.4 units. Then swell to 18 units in the course of 2 to 5 minutes.

The immature eggs of *Fissurella* sp., *Eurythoe* sp., and an *Amphitrite*-like worm behave as do those of the palolo.

It was often noticed that if segmenting eggs were placed in NaOH different blastomeres were entered at very different times by the alkali. Thus the resistance of each individual blastomere is specific and on division the daughter cells may become perfectly distinct from each other so far as permeability relations go. Whether such differences are related to the hereditary potencies of the individual blastomeres was not determined.

It will be seen from the foregoing observations that several types of eggs may be distinguished as regards penetration of NaOH and cytolysis, viz: (1) Eggs in which cytolysis and color change are both simultaneous and rapid (*Toxopneustes* and sea-urchin eggs in general); (2) eggs in which cytolysis and color change are simultaneous but gradual (*Eunice* and other annelids); (3) eggs in which color change may occur *before* cytolysis (*Holothuria floridana*, immature eggs).

In addition to the above: (4) eggs in which the color change occurs after cytolysis (*Cumingia*, observed at Woods Hole, Massachusetts).

In *all* types ammonia and the amines enter instantly, long before cytolysis occurs.

Only one egg thus far examined (that of *Cumingia tellinoides*) belongs to type 4, and one egg only (that of *Holothuria floridana*) to type 3. In other cases penetration of NaOH and swelling are simultaneous.

Leaving aside for the moment the egg of *Holothuria*, which forms an exception to the rule that cells are visibly affected before or at the moment of entrance of NaOH, let us analyze the results obtained with eggs of types 1 and 2.

The rapid color change in ammonia shows how small the concentration of OH ions need be to affect the red-stained granules. We may assume, then, that if even a trace of NaOH entered the color change would be rapid. Since practically all marine eggs remain quite unchanged in size and in color (of the red-stained granules) in NaOH solution for some time and only after a definite interval begin to swell and to change color relatively rapidly, we may conclude that the NaOH can only force an entrance after fundamentally changing the normal nature of the cell-surface. The manner in which cytolysis is connected with such a change in the cell-surface may be conceived as follows:

The NaOH combines with substances, probably with proteins at the surface of the egg, forming a compound through which it may pass; *i. e.*, the surface is made permeable to NaOH. This compound must also allow the salts of sea-water to pass readily; the egg is likewise made permeable to the salts of sea-water.[26] Osmotic equilibrium is upset and the egg absorbs water and salts. Breaking up of the granules is due largely to the increase of water in the egg. All marine eggs swell when placed in fresh water and when the increase in volume reaches a certain value the contained granules break down; true cytolysis results. The breaking-up of the granules by distilled water is obviously not due to any chemical substance, such as alkali, entering the egg. An increase in permeability might result in swelling without the entrance of the cytolytic substance. Such is the case when *Cumingia* eggs are placed in NaOH.

However, I am inclined to think that the presence of a sufficient number of OH ions within the egg may aid in the breaking down of the granules and that this breaking down increases also the degree of swelling of the egg. Cytolysis in *Holothuria* appears to be largely of this type, since here NaOH enters before the increase in volume begins.

From this point of view both theories of cytolysis contain an element of truth. Swelling of marine eggs is due both to an increase in permeability of the surface and also to the breakdown of lipoid or protein granules within. The latter tends to increase the swelling pressure or the osmotic pressure of the egg, but is secondary to the increase in permeability of the surface.

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[26] McClendon (Am. Jour. Physiol., vol. 27, p. 240, 1910) finds the electrical conductivity in sea-urchin's eggs to be greatly increased after cytolysis, a result indicating ready passage of the salts of sea-water.

IX.

PHYSIOLOGICAL STUDIES ON CERTAIN PROTOZOAN PARASITES OF DIADEMA SETOSUM.

By MERKEL H. JACOBS,
Instructor in Zoology, University of Pennsylvania.

PHYSIOLOGICAL STUDIES ON CERTAIN PROTOZOAN PARASITES OF DIADEMA SETOSUM.

By Merkel H. Jacobs.

INTRODUCTION.

It has been shown by the author and others that different species of protozoa have certain physiological characteristics, often almost as striking as their morphological ones, and which are probably of considerable significance in the interpretation of their habits of life and their relation to their environment. The study of such characters in the past has been greatly neglected on account of the general belief of biologists that they are exceedingly inconstant and subject to modification by external factors and therefore of little value in an understanding of the fundamental nature of the organisms possessing them. Previous attempts to detect characteristic physiological differences between different forms have for the most part been confined to organisms whose general habits of life are so dissimilar as to leave room for the objection that the differences observed may have been due merely to the effect on the animals of the different environments to which they have been accustomed and not to any innate peculiarities of the organisms themselves.

It occurred to the author as desirable to test, if possible, a series of forms which naturally live under essentially the same environmental conditions, and which may be assumed to have done so for many past generations, in order to see whether they show greater likenesses than a number of forms selected at random, or whether each has preserved its individuality in spite of the similarity of its environment. In the case of a number of parasitic protozoa living together in the same organ of the same host, we have a favorable opportunity for such a study. Even here we can not assume that each form has exactly the same environment, but, there can be no question that this environment is far more similar than that of a number of selected free-living forms. Furthermore, in order to become parasites of the same host the nature of the forms in question must have been more or less changed. In the case of intestinal parasites, for example, it would be necessary for them to acquire a resistance to the digestive juices of the host and to become accustomed to a more or less anaerobic habit of life, etc. Such changes would necessarily be in the same direction for all of them. If, therefore, a number of parasitic forms, which have had to meet and adapt themselves to the same new conditions and live under them together for thousands of generations, still show as characteristic physiological differences as free-living forms, it is good evidence of the fundamental nature of these differences. Is such the case? The present paper is a preliminary consideration of this question. The work on which it is based was performed at the Tortugas Laboratory of the Carnegie Institution of Washington during the summer of 1910.

MATERIAL.

The material used in these experiments consisted of four species of ciliate protozoa obtained from the alimentary tract of the large black sea-urchin, *Diadema setosum*, which can be procured in practically unlimited quantities from the coral reefs about the Tortugas. This form is particularly favorable as a source of material, not only on account of its abundance but on account of the high percentage of infection shown by it with respect to the protozoa in question. Out of over 100 adult Diademas examined during the progress of the work, not a single one failed to show one or more kinds of protozoa in the intestine, which often fairly swarmed with them. Doubtless the large size of the intestine, its pouch-like recesses in which stagnation of the food can occur, and the character of the food itself are factors favoring the presence of protozoan parasites or, perhaps more correctly, protozoan commensals, since none of the forms in question seem to be injurious to the host or to live at its expense, except in so far as they use up a small portion of its practically unlimited supply of food.

The forms studied were of four kinds. Since apparently none of them have been previously described or named, they are for convenience designated in the account that follows by the letters, *A*, *B*, *C*, and *D*. Of the four, *B* is the most regular in its occurrence, having been found in 100 per cent of the adult Diademas examined. It is a large, heterotrichous form allied to *Metopus*, with a hook-like anterior end and showing considerable individual variation in appearance.

The form next in abundance is *C*, which is found in about 75 per cent of the cases. It is a medium-sized, holotrichous form, elongated in shape, with the anterior end of the body transparent and longitudinally striated and the remainder filled with refractive spherules. A nucleus is visible about the middle of the body and no mouth opening can be detected.

The next form in importance is the one designated as *D*, which is found in 40 per cent of adult Diademas. It is flat and leaf-like in form, with the body slightly concave on the ventral and convex on the dorsal surface. The one margin of the body is almost straight and the other curved, giving the animal an asymmetrical shape. At the posterior end is a spur-like projection. Evidently this form is allied to the holotrichous genus *Dysteria*.

The form which occurs in the smallest number of cases (33 per cent) is *A*. When present at all, however, the number of individuals is generally very large. In size it is rather small and in shape elliptical and flattened.

Very little could be made out of its structure, owing to its small size, rapid movements, and its peculiar tendency to disintegrate with almost explosive suddenness when kept under observation on a microscopic slide. In addition to these four characteristic forms, several other ciliates were observed more rarely, and on one occasion an *amœba*. They were not found frequently enough, however, to be considered in the experimental part of the work. So far as the present observations go, the important group of flagellates appears not to be represented by parasitic forms in *Diadema*.

The parasites of *Diadema* seem to occur exclusively in the alimentary tract, none being found in the body-fluid so long as the intestine is uninjured. In the alimentary tract itself the number is not the same in the different regions, some parts being more favorable than others. The greatest numbers are found in the distended pouches of the second, or upper, coil of the intestine. In the lower coil they seem to be almost or entirely absent, even when present in great abundance elsewhere. It is interesting to note in this connection that free-living forms (*e. g.*, *Foraminifera*, etc.), which are taken in with the food, are invariably dead before the second coil is reached. The rectal portion of the intestine also shows very few protozoa, form *D* being the one most frequently encountered there, though *B* and *C* may occur rarely and in small numbers. On several occasions cysts, possibly of *D*, were found in this region. It seems quite probable that cysts thus formed may pass out and eventually infect other Diademas. It does not always happen that the different protozoa present show the same distribution, thus, *A*, for example, may be quite absent in some of the pouches and present in others, while *B*, *C*, and *D* show similar irregularities. It is usual to find several of the forms together, but there are no constant combinations; any form may occur associated with any of the others.

The above remarks concerning the abundance and distribution of the parasites apply only to adult Diademas. As might be expected from our knowledge of other animals, young individuals contain either no parasites at all or at most but a few. The youngest ones examined (0.5 inch in diameter) contained nothing. Somewhat older ones (1.5 inches in diameter) contained sometimes nothing at all, sometimes large numbers of a form not found in any of the adults examined, and in a few cases small numbers of *C* and *D*. It is rather striking that form *B*, which appears to be present in all adults, should be so constantly lacking in younger individuals. Whether this is because conditions for its existence are unsuitable or simply because no opportunity for infection has occurred could not be determined.

A search for the parasites in question was also made in sea-water and in other animals, related and unrelated to *Diadema*. The only situations in which any of them were found was in the alimentary tract of other sea-urchins, where they may occur in varying numbers. *B*, for example, was found rarely in *Toxopneustes* and *Echinometra*, and *C* on a number of occasions in *Toxopneustes*, while *D* seems to be present in every adult *Toxopneustes*, and rarely in *Echinometra*. *A* was never found outside of

Diadema. A number of specimens of *Hipponoë* were examined for protozoan parasites without results.

METHODS.

In comparing the physiological nature of the forms in question certain characters were chosen which could be expressed quantitatively and the animals examined with respect to them. Those selected all had to do with the length of time the organisms could maintain their normal activities under various conditions differing from those to which they were accustomed. The method adopted was to place the animals under the new conditions and note the time that elapsed until all visible movements ceased. In the case of ciliates this time corresponds very closely with that at which irreparable injury, resulting in death, has been done to the cell, and therefore furnishes a more or less accurate criterion of the general vitality of the organisms.

In the present experiments the number of individuals studied was always large and the range of variation within the species small, and therefore it was found possible to choose, as the point for comparison, the time at which all the individuals of the given species had been rendered motionless. This point was easy to determine and the general agreement in the results obtained on successive days by the use of the above method showed it to be sufficiently accurate in a case, such as the present one, where it was desired to express the relative and not the absolute resistance of the four forms in question. Most of the experiments were repeated a number of times with material obtained from different sources, so as to eliminate accidental errors. The characters, in respect to which the different forms were compared, were as follows: (1) Ability to live outside the body of the host; (2) ability to live in the body-fluid of other related animals; (3) length of life of the parasites after the death of the host; (4) resistance to CO_2; (5) resistance to H_2S; (6) resistance to the decomposing proteid substances; (7) resistance to H_2SO_4; (8) resistance to KOH.

EXPERIMENTS.

ABILITY OF THE PARASITES TO LIVE OUTSIDE THE BODY OF THEIR HOST.

It is well known that protozoan parasites show a varying ability to live apart from their host. In general, the less specialized the parasite and the looser the relation between it and its host, the longer it may be expected to live under conditions other than the natural ones. The forms in question are not highly specialized and, as might be supposed, are for the most part able to live for a considerable time outside the intestine of *Diadema*, though so far as these experiments go, a continued existence of this sort is in no case possible. The point of particular importance, however, from the standpoint of the present investigation, is that the four forms studied show marked differences in behavior when removed from the body of their host. If a number of food pellets from the intestine of a *Diadema* be shaken up with a little fresh sea-water, none of the parasites obtained in this way is at first visibly injured. If, however, a few cubic centimeters of the mixture be placed in a covered glass dish which prevents evaporation but which, being only partially filled, does not prevent contact of the liquid with the air, it will be seen that all of the animals present will die after a length of time which is fairly constant for each species. The first one to disappear apparently always is *A*, which is no longer present after 2 to 3 hours.

It may be mentioned that no way of keeping *A* longer than this in artificial cultures was found except to place a relatively large quantity of the intestinal contents in a test-tube with the addition of only sufficient water to keep it moist, in which case the animals retained their activity somewhat longer. *D* is next to *A* in respect to its behavior in sea-water culture, and usually is dead at the end of 24 hours, though a few individuals may survive several hours longer. *C* does not differ very markedly from *D*, but is somewhat more resistant, living on an average 30 hours or a little over. *B* is by far the most resistant form, often surviving and continuing active for 2 or 3 days. The relative ability of the four forms to live outside the body of their host may therefore roughly be expressed by the ratio 1 : 24 : 12 : 10.

To determine to what extent the injurious effects of removing the parasite from their host were due to the action on them of the oxygen of the air, a parallel series of experiments was tried in which oxygen, so far as possible, was excluded. This was done by shaking up the food pellets from the intestine with sea-water from which the air had been removed by boiling (the concentration of salts being kept constant by the addition of distilled water) and immediately placing the liquid in dishes which were completely filled, covered, and sealed with vaseline to exclude the air. Such treatment was found not materially to alter the length of life of any of the forms except *B*, which in one such experiment lived for 7 days, the longest time for which it was found possible to keep any of the forms alive by any of the methods employed. *A*, *C*, and *D* died in about the usual times, the life of *A*, if anything, being slightly shortened and that of *C* slightly lengthened. In no case except that of *C*, however, were the differences significant.

ABILITY OF THE PARASITES TO LIVE IN THE BODY-FLUID OF TOXOPNEUSTES.

Early in the course of these experiments it was observed that the forms in question live in cultures made with the body-fluid of *Diadema* about the same length of time that they do in sea-water. This is not surprising, since the osmotic properties of the body-fluids of sea-urchins and sea-water are about the same. A point of some interest seemed to be whether the body-fluid of a nearly related genus of sea-urchins would be as favorable as that of the host or as sea-water, or whether there might be present in it in addition to the salts of sea-water certain other substances of more specific nature which would have a toxic effect. To determine this point, therefore, experiments were made, but unfortunately they were not very extensive. One such experiment may be mentioned. *A*, *B*, and *C* obtained from *Diadema* were placed in a small quantity of the body-fluid of *Toxopneustes*.

A was found to be dead at the end of 1.5 hours, *B* in 3 hours, and *C* was living after 15 hours, but dead in 24. In a control culture in which the body-fluid of the original host was used in place of that of *Toxopneustes*, *A* lived about 2 hours, *B* was still active at the end of 24, and *C* died somewhere between 15 and 24. It will be seen, therefore, that *A* and *C* are less affected by the change than is *B*, which normally lives 10 to 20 times as long in the body-fluid of its own host as in that of *Toxopneustes*. Unfortunately *D* was not available at the time when this experiment was performed. The converse of the above experiment, namely, the subjecting of parasites obtained from *Toxopneustes* to the body-fluid of *Diadema*, was also tried with similar results. In this case only *D* was available, it being the only one of the four forms found in quantity in *Toxopneustes*. In the control culture in which the body-fluid of their own host was used the animals were normal after 15 hours; in the body-fluid of *Diadema* they were dead in 2 hours.

The injurious effects of transferring the parasites to the body-fluid of an animal other than their host are almost certainly not due to physical (*e. g.*, osmotic) differences between the body-fluids of the two animals, since they may be transferred with impunity from either host to sea-water, the body-fluid of sea-urchins having approximately the same osmotic properties as the latter. Furthermore, in a number of experiments in which the concentration of the liquid surrounding the parasites was purposely suddenly altered, the forms in question were shown to be quite resistant to changes of this sort. It may be assumed, therefore, that chemical rather than physical differences in the two body-fluids are responsible for the death of the animals. It is rather interesting that different races of the same species are found adapted to different hosts and apparently can not be suddenly transferred from one to the other. Where adaptive characters such as these are concerned, we can look for a considerable amount of variation within the species. But in the case of non-adaptive characters, such as some of those mentioned in the succeeding experiments (resistance to H_2S, H_2SO_4, etc.), it was found that the properties of form *D*, for example, were practically the same whether it was obtained from *Diadema* or *Toxopneustes*, and consequently that such characters are fairly constant for the species.

LENGTH OF LIFE OF THE PARASITES AFTER THE DEATH OF THE HOST.

If a dead *Diadema*, either opened or unopened, be covered with sea-water and allowed to stand in a warm room, the number of parasites present soon begins to diminish and after a variable time all disappear. Sometimes in as short a time as 12 hours none are to be found, though in other cases they may be active for 24 hours or more. In no instance, however, do they seem to persist more than 30 hours at the room temperature of the Tortugas laboratory (85° to 95° F.). They usually disappear in the order, *D*, *A*, *C*, *B*. In one series of observations *D* was dead in 12 to 15 hours, *A* in a little less than 20, while *C* and *B* survived somewhat over 24. While the absolute time of disappearance differs in different experiments the relative order for the four forms seems to be quite constant.

It will be seen, therefore, that in respect to their ability to live in the body of the host after the latter's death, just as in many other respects, they show certain characteristic physiological differences. The relative length of life of the four forms is roughly 1.5 : 2 : 2 : 1. Just what factors cause the death of the parasites under these conditions is not certain, but probably the products of decomposition present play an important part. As will be shown later, some of these substances are markedly toxic, though each of the four forms is affected differently by them and the immediate cause of death may perhaps be different in the different cases.

EFFECTS OF CO_2 ON THE PARASITES.

Carbon dioxide certainly occurs normally in the intestine of *Diadema* and therefore small quantities of it can not be markedly injurious to any of the four forms in question. With large quantities the case is different, all being killed in a fairly short time, though the four forms are by no means alike in their resistance. If a drop of fluid containing them be exposed to a continuous stream of the gas in a gas-chamber and kept under constant observation, it will be seen that *D*, the least resistant form, is rendered motionless in 3 or 4 minutes; *A* comes next, the time required for it being 5 to 6 minutes; *C* remains active for about 15 minutes, and *B* for from 45 to 60 minutes. The ratio of resistance is therefore roughly 1.5 : 15 : 4 : 1; *B* being the most resistant form and *D* the least. The same order of resistance was found to hold if instead of subjecting the animals to a stream of gas they were placed in a relatively large quantity of sea-water charged with CO_2 by means of a "sparklet" bottle.

EFFECT OF H_2S ON THE PARASITES.

Hydrogen sulphide begins to appear in the intestine of *Diadema* in considerable quantities soon after death. The vapors given off on gently warming a little of the intestinal contents taken 12 hours after death darken lead acetate paper very perceptibly, and after 24 hours the reaction may often be obtained without the application of heat at all. As this substance might be suspected of playing a part in causing the disappearance of the parasites from the intestine of the host after death, it was of interest to examine its effects on the four forms in question. As might be expected, it proved to be markedly toxic to all, but the different forms resisted it in pronouncedly different manner, and, furthermore, the order of resistance is different from that shown to CO_2. *C*, for example, which is next to the most resistant form to CO_2, is killed first when a current of the gas is passed through the gas-chamber containing the animals, the time required being only 1 to 2 minutes; *D* comes next, with a period of about 3 minutes; *A* lives 3 to 4 minutes, and *B* 10 to 15. The ratio of the resistance of the four forms is therefore approximately 2.5 : 8 : 1 : 2.

EFFECT OF DECOMPOSITION PRODUCTS ON THE PARASITES.

In an attempt to throw light on the cause of the disappearance of the parasites after the death of the host, the effect on them of the direct products of the decomposition of the tissues of *Diadema* was tried. The method used was to allow fragments of *Diadema* tissues to decay in the water for 3 days at room temperature; 5 drops of the resulting infusion were then added to 10 drops of the fluid containing the animals. The results were most surprising. *B*, which had hitherto proved the most resistant form in most of the experiments, was killed almost instantly—certainly in less than 5 seconds; *A* lived from 10 to 60 seconds; *D* 20 to 25 minutes; and *C* as long as 2 hours. *C*, therefore, which is less resistant than *B* to most conditions, is in this case many more times resistant. The ratio for the four forms is roughly 7 : 1 : 1,500 : 250. These figures seemed so remarkable that the experiment was repeated several times, but always with essentially the same results.

THE EFFECT OF H_2SO_4 ON THE PARASITES.

A limited number of experiments were tried with this substance. It was necessary first to determine the proper concentration, since if the solution is too strong death occurs so quickly that its time can not be accurately observed, and if too weak it occurs so late that other factors have time to complicate the results. A series of preliminary experiments showed that a favorable proportion is 1 drop of N/10 H_2SO_4 to 5 drops of the fluid containing the organisms. Of course, care had to be taken that the acid was immediately mixed thoroughly with the culture medium, otherwise the organisms were not all subjected to exactly the same conditions. Treated in this way, the four forms showed striking differences: *A* had a very low resistance, being killed in about 5 seconds; *D* was killed in less than 30 seconds, but the exact time (somewhere between this and 5 seconds) was not noted; *C* died in about 30 seconds; while *B* survived 1.25 hours or 900 times as long as *A*. This is seen to be a surprisingly high resistance to acid when it is remembered that *Paramecium* and many other fresh-water forms are killed almost instantly by exposure to N/500 H_2SO_4. Summarizing the results of the observations on the resistance of the four forms to H_2SO_4 we have the ratio, 1 : 900 : 6 : $<$ 6.

EFFECT OF KOH ON THE PARASITES.

This substance used in the same concentration as H_2SO_4, as might be expected from results obtained with other animals, is on the whole much less injurious. *A* (the least resistant form) was found to be killed in about 2 minutes, *D* in somewhat less than 45 minutes, *B* in about 1 hour, and *C* in 3 to 4 hours. It will be noted that while *A*, *C*, and *D* are much more resistant to KOH than to H_2SO_4, *B* is actually less resistant. The ratio for the four forms is roughly 1 : 30 : 100 : 20.

CONCLUSIONS.

The general results of the experiments performed is to show surprising differences in the resistance of the parasites of *Diadema* to various unfavorable conditions. In some cases the most resistant form may live several hundred times as long as the least resistant one. *B* is 900 times as resistant to H_2SO_4 as *A*, and the difference between *B* and *C* with respect to products of decomposition is still more striking. Furthermore, a form which is strongly resistant to one condition may be only feebly so to another, and vice versa. For example, *B* is 8 to 10 times as resistant to H_2S as *C*, but 1,500 times less resistant to certain of the products of decomposition of *Diadema* tissues. *C* is more resistant to CO_2 than *A* or *D*, but less resistant to H_2S. *A*, *C*, and *D* are 24 to 450 times more resistant to KOH than to H_2SO_4, while *B* is somewhat *less* resistant. Other similar facts could be mentioned.

Comparing all of the results obtained, it is therefore seen that the similar habit of life of the four forms in question has not brought about physiological similarity except in certain adaptive characters which are a *sine qua non* for continued existence in the same host (*e. g.*, ability to resist the digestive juices of the latter, etc.). In other respects they are just as different as almost any four free-living forms that might be selected and so far as the evidence of these experiments goes it seems to show that the physiological characters of an organism are not merely the result of its environment, but may be as fundamental and characteristic as its morphological ones.

X.

ORIGIN OF THE ELECTRIC TISSUES OF GYMNARCHUS NILOTICUS.

BY ULRIC DAHLGREN
Professor of Biology, Princeton University.

Nine plates and nine text-figures.

ORIGIN OF THE ELECTRIC TISSUES OF GYMNARCHUS NILOTICUS.

BY ULRIC DAHLGREN.

In the seven types of electricity-producing fishes the exact development by which the electric organs and tissues are produced during the creature's life is known in only two, leaving the other five unknown. It also happens that the two forms which have been studied as to the histogenesis of their electric organs are the only two elasmobranch fishes among the seven, so that we have not as yet seen how the remarkable electric tissues in *Malopterurus*, *Gymnotus*, *Astroscopus*, the mormyrids, and *Gymnarchus* are developed. Also, of the five teleost types we know the structure of the full-grown electric organs in all of them pretty well, except in *Gymnarchus*. This fish is found in Africa and has been rather rare, so that but two workers have published observations on it, both of them a long time ago and from poorly preserved material.

It was, therefore, with great pleasure that the writer came into possession of some embryos of this rare form through the kindness of Dr. J. Graham Kerr, Dr. Arthur Shipley, and Dr. Richard Assheton, to whom he wishes to express his most sincere thanks. This material was collected in Africa by Dr. Samuel Budgett some years ago and was in most excellent condition, owing to the great care and skill with which Dr. Budgett put it up and cared for it. The collecting was done unflinchingly and faithfully, under conditions of hardship and sickness that few white men could stand, and Dr. Budgett lost his life from exposure and illness incurred in part by this work. A full account of his trip and of the scientific results should be read in the Budgett memorial volume issued by Dr. Shipley, Dr. Kerr, Dr. Assheton, and others in 1907, through the Cambridge University Press (24).[1]

It is somewhat unfortunate that the structure of the electric organs in the adult fish could not be worked up at the same time that this paper was written, but the writer has material on the way from Khartoum and hopes to publish a second paper shortly.

But three papers have been published on the electric organ of this interesting fish, one by Erdl (15) in 1847 and another by G. Fritsch (19) in 1885. Rüppel's publication on the subject could not be found, but Fritsch states that Rüppel mentioned the peculiar structures which we are considering, and so he stands at present in the writer's knowledge as the first one to have seen and reported to science the electric organs of this fish, although he was in doubt as to their significance. Erdl used a specimen which was so poorly preserved and so soft that when he cut the animal across its body, the electroplaxes and connective tissue ran out of the muscle in which they are embedded. Nevertheless Erdl stated that it was probably an electric organ, coming to this conclusion by a comparison, of such features as he could make out, with the structures found in the tails of the other electric fishes, as *Raja*, *Mormyrus*, and *Gymnotus*.

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[1] The figures in parenthesis refer to the literature cited, p. 193.

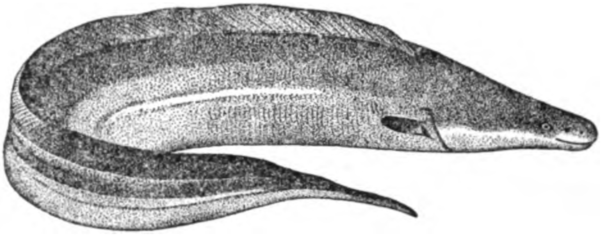

FIG. 1.—General view of *Gymnarchus niloticus*. (Drawn from a lantern slide made from a figure in Jordan's "Guide to the Study of Fishes," New York, 1905.)

Fritsch (19) in 1885 worked on better material and gave a more complete account of the anatomy of the organ, especially of the histology of the electroplax; but he came to the rather strange conclusion that it was not an electric organ at all, assuming the erroneous position that, on account of the large blood-supply, the organ acted in some way as a storage for oxygen during the period of hibernation made necessary by the drying of waters at certain seasons. Fritsch was also mistaken in calling the fibrous contents of the electroplaxes "connective tissue." He went a great deal farther than Erdl, however, in describing the gross anatomy of the electric organs and surrounding tissues.

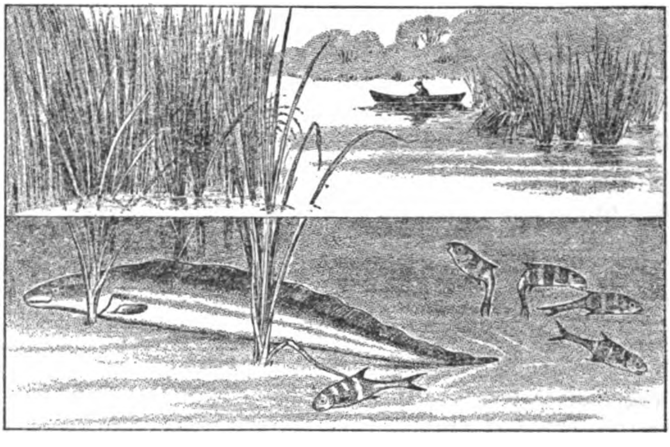

FIG. 2.—Scene drawn from descriptions to illustrate the habitat of *Gymnarchus* and its manner of swimming. (Drawn from lantern slide made from a drawing, by Bruce Horsfall.)

Not having full-grown material, the writer must rely on Fritsch's figures and descriptions for the adult gross anatomy, although the oldest embryos (about 42 days) used for this paper represent practically adult material so far as the histology of the electroplaxes is concerned. A short résumé of the adult anatomy will make a good basis for the embryological descriptions to follow.

The fish (see text-fig. 1) is a mormyrid of elongate form, so much so as to make it almost eel-shaped, although not quite so much so as the *Gymnotus* of South America. It possesses an extensive development of the dorsal fin, which extends from forward on the neck to within a short distance of the tip of the tail, where it suddenly stops, leaving the tip of the tail naked of fin; whence the name of the fish, *Gymnarchus niloticus*. This heavy fin, well provided with a series of lateral ray-muscles, is used extensively by the fish as a means of propulsion, by holding the body straight and stiff and causing a series of lateral undulations to pass from behind forward, thus driving the body backward (see text-fig. 2) or, from front to rear, which causes the fish to move with its head forward. I have not heard, but I presume that in moments of unusual effort the animal can swim by means of the common sinuous body-movements used by other elongate fishes, as is well illustrated in the eel.

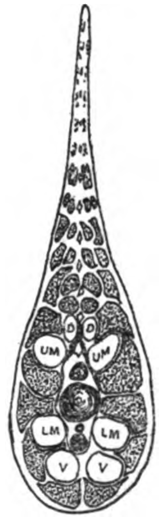

FIG. 3.—Section through mid-tail region of body of an adult *Gymnarchus*, showing position of the eight electric spindles and their relations to surrounding muscular and bony structures. *D*, dorsal spindles; *U.M.*, upper median spindles; *L.M.*, lower median spindles; *V*, ventral spindles. (After Fritsch.) X unknown.

The posterior tip of the creature's body is interesting. As has been mentioned, this end is free from the fin for some distance (see text-fig. 2). Also it is round in section and ends bluntly. When swimming backwards the animal uses it like a finger to feel its way. The peculiar round and blunt end may be explained by the fact that this tip contains the largest and best-developed portion of the electric organ, which fills the lateral parts of the body at this point almost to the exclusion of the ordinary muscle.

As Erdl and Fritsch have described, the electric organ consists of eight long "tube-like" or cylindrical structures, four on each side, embedded in the muscle tissue as close to the median bony parts as a little connective tissue in between will permit. Four of these are present on each side (see text-figs. 3 and 4), and they may be called in order from above downward, the ***dorsal***, the ***upper middle***, the ***lower middle***, and the ***ventral*** cylinders, or spindles, of the electric organ. In a section cut through the body at a point midway between tail-point and anus (see text-fig. 3) the dorsal spindles are to be found, just above the union of the neural spines of the vertebræ, and set closely together with only the dorsal spine and some connective tissue between them. The upper median spindles are more widely separated by the neural canal and its contained spinal cord. This second pair of spindles are at the level of the upper part of the spinal cord. The lower median spindles are found much below, on each side of the ventral processes of the vertebra. They lie at about the level of the caudal artery and almost as widely separated as the upper median pair. Lastly, the ventral pair of spindles are found just below the latter pair and slightly below the level of the caudal vein, which lies between them. They are separated a little less than the lower median pair by the narrower bony structures at this point.

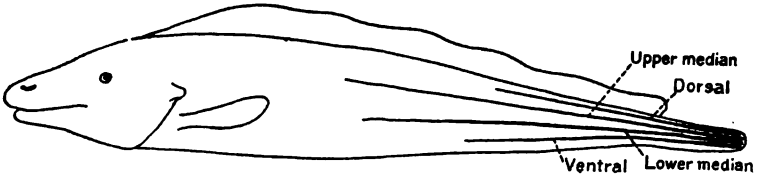

FIG. 4.—Diagram showing longitudinal position and extent of electric spindles in *Gymnarchus*. Those of one side only can be shown in figure.

The four spindles are largest in diameter in the tail, especially out in the thick, finger-like, naked extremity. From this part they taper to a smaller size as they go forward in the body and they finally become thin and end at points in the neighborhood of the anal opening (see text-fig. 4). All are not of equal length. As Fritsch has shown, the dorsal organ is the shortest and extends for about 20 cm. in a fish of 89 cm. length. The ventral pair reach for about 5 cm. further, or 25 cm. in length in a fish of the same size. The two median spindles reach for about 40 cm. from the tip of the tail.

Each spindle is marked off clearly from the neighboring muscle, and other tissues which are found next to it, by a distinct connective-tissue covering. In my largest specimen, which is a young fish of 40 days, this is well shown and is exactly like other dividing connective-tissue sheaths that surround the various muscle divisions. Like them, it often contains pigment cells which show golden-brown pigment granules.

The important contents of these spindles are alternate, cylindrical segments of a denser, deeper-staining, muscle-like substance, the electroplaxes; and a connective tissue of jelly-like, grayish transparence which in all ways appears to be similar to the "electric connective-tissue" found between the electroplaxes in the other electric fishes. In this tissue are found the blood-supply, which is largely in contact with the ends of the electroplaxes, principally the anterior end; and the nerve-supply of thick, medullated fibers which run towards, and are attached to, the posterior ends of these organs. In this case, according to both Pacini's law and the fish's relationship to *Mormyrus*, the direction of the current at time of discharge should be from tail towards the head. I have been unable to learn, from the literature of travelers and scientific collectors and observers, if the electric discharge is strong enough to be felt by the hand. Budgett does not mention it, and no other does, so I conclude that it is not a strong shock and that the organ must be classed with the weak electric organs, as is the case with its relatives, the various mormyrids. The natives of Africa are much afraid of the creature, especially at nesting time, and one of its Arabian names, "Abu rhad" meaning "father of thunders," might seem to indicate perceptible electric powers.

The embryos and young fishes put at my disposal by Dr. Kerr and Dr. Assheton were five in number, and of these three were the suitable stages from which this paper was worked out. The significant development of the electric tissues in *Gymnarchus* takes place between the ninth day of embryonic life, at which time the embryo possesses a fully formed and complete musculature in the tail with no sign of an electric organ, and the fortieth day of development, at which time it can be seen that the embryo has developed its electric organs, out of a certain part of the previous musculature in the tail, to a degree that shows the farthest advanced electroplaxes in a practically adult condition.

The most interesting and critical stages in this metamorphosis of muscle into electroplax appear to take place within much closer limits, and stages from the eleventh to the fifteenth day would include them. These significant changes have been studied and drawn principally from an embryo 12 days of age, fixed in sublimate-acetic, and showing the changes very much to my satisfaction. A point of interest and importance in this study is that, in earlier embryos, the myotomes and electric spindles are youngest, least developed, and growing fastest in the posterior part of the body or nearest the tip of the tail; while in older embryos and in the adult the greatest, most complete, and most characteristic development of the electroplaxes is to be found in the end of the tail or at the posterior end of the spindle. Thus the adult structures in the anterior part of the spindles represent a somewhat inferior and less complete change of the muscle tissue into electric tissue than the posterior parts of the same organs do.

The same importance attaches to the fact that the rates of development of the several spindles seem to vary. The lower median spindle starts first to differentiate, extends farthest forward in the body, is larger than the others when developed, and during early development is always in advance of the corresponding parts of the other spindles. The upper median spindle closely follows the lower. The ventral spindle is much behind the two median ones, while the dorsal spindle represents the latest and weakest development and is shorter than any of the others. These facts have made it possible in the present study to get many stages of development from very few embryos.

STUDIES OF AN EMBRYO NINE DAYS OF AGE.

This little fish was 26 mm. in length and, while the egg-membrane had been ruptured and cast away, the animal was still forced to remain in its nest because of its huge, elongate yolk-sac, still unabsorbed, and because of its otherwise undeveloped organs of alimentation, locomotion, etc. The posterior part of the body was carefully cut into four portions (see text-fig. 5) to be sectioned as follows: First, from in front of the anus to a point about 26 vertebral segments posteriorly. The last three segments of this portion were sectioned transversely and serially (region A_1) while the remaining anterior part was sectioned vertically and longitudinally (region *A*). Another portion of 19 vertebral segments further caudad was removed and its posterior two segments sectioned transversely (region B_1), while the anterior part was cut as before in vertical, longitudinal sections (region *B*). A third part, of 18 more vertebral segments, was treated in the same way, except that no transverse sections were taken and the entire piece was cut vertically and longitudinally (region *C*), while the remaining portion or tail-tip was cut serially in transverse sections and forms a series (region *D*).

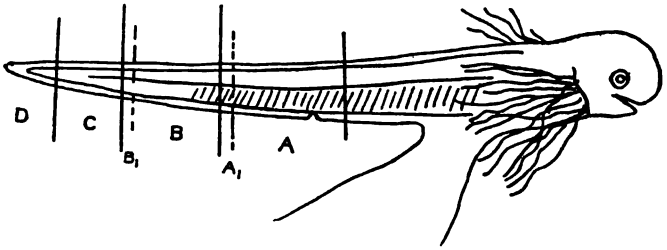

FIG. 5.—Outline of body of an embryo of *Gymnarchus* nine days old. Transverse lines and letters indicate parts sectioned for study. For explanations see text. (Copied from Assheton in "The Work of John Samuel Budgett.") × 5.

Figure 1, plate 1, shows a transverse section through the body a rather short distance from the extremity of the tail or at a point where we are sure that the electric organ will be well developed a little later in life. It may be thought that such a section could be taken for study to better advantage in a more anterior position on account of the earlier anterior development just discussed, but it must be taken into account that the tail segments are being added and are still growing rapidly at this age, that they are very short and very crowded, and therefore the location of this section is, in reality, fairly well forward in the future electric spindles. Conditions were much the same in region *C*.

This section shows a good development of muscle fibers as indicated by the shaded area. As is usual in vertebrates, the most advanced stages of muscle-cell development are to be seen at the lateral periphery of the myotome. Here, at the point indicated in figure 1, plate 1, by the dotted line, a layer of the outer muscle cells, two or three deep, has acquired an average of about 26 myofibrils (an average of 14 counts). These are grouped in one (fig. 2, plate 1, *B* and *C*) or sometimes two bundles in the cell (fig. 2, plate 1, *A*) and their correct spacing and their thickness and staining power indicate muscle cells of normal development and good functional activity. The remaining and inner muscle cells of the myotome, forming its larger bulk, show, as one examines them successively farther inward (toward the median line), a series of earlier stages, until at many points on the inner edge of the myotome the smallest cells are seen with large nuclei and no myofibrils at all (fig. 2, plate 1, *C*). These youngest cells are particularly abundant at the dorsal and ventral edges of the myotome.

From what we know of the position of the future electroplaxes and their relation to the muscle-masses, we can be sure that it is from the inner edge of the myotome that the electric tissue is to come, and a close scrutiny of the cells which form this edge shows that at two points only is there any indication of such a development.

One of these points, marked with a circle (⊕) in figure 1, plate 1, is where the two myotome segments (dorsal and ventral) meet and close in against the notochord. Here we see, in some of the sections, several especially large and strongly developed fibers, somewhat detached from the rest of the myotome and resting against the vertebral disk. Several reasons exist, however, why these fibers do not represent the future electric organ. First, they are in the exact median position which remains constant during growth and in which no electric tissue is to appear. Second, they are very short and are not attached to each other longitudinally, as other fibers are, by means of connective tissue, but are attached to the bodies of the future vertebra. They may be called the vertebral fibers.

A second point can be seen where some muscle-tissue shows unusual development. At this point (marked with a circle (○) in fig. 1, plate 1) are several muscle cells which show from 10 to 18 myofibrils each, and a degree of development almost equal to the cells in the outer layer. These cells are represented as seen under high magnification in figure 3, plate 1. They are, I believe, destined to be the future electric cells, several of which will unite and form one of the electroplaxes of the lower median spindle of the electric organ. I base this assertion only on their position and their somewhat advanced development as muscle fibers, for they show at this time no indication, other than their size, of developing into electric tissue.

Their position is slightly too far dorsal for the lower spindle, in an adult *Gymnarchus*, but when we examine the 12-day-old and 42-day-old stages, we find that the normal growth of the muscle-mass will carry this point to exactly the proper position for that spindle to lie at, in the grown fish.

An important point in studying these cells in figure 3, plate 1, is to note that they are separated from the rest of the myotome and from one another by other muscle cells of weak or of earlier development and containing only a few myofibrils. These weaker cells, and even some of the more advanced ones, are destined to degenerate during the development and growth of the electroplax. In figure 3, plate 1, 7 cells are present that will probably take part in the formation of the electric spindle at this point. Some of them are contiguous and others are widely separated.

In figure 4, plate 1, we have a fortunate longitudinal section from the adjacent region of this same embryo. The section shows in longitudinal view, and under low magnification, the same group of muscle cells marked with the circle (○) at both ends. Also, it shows a portion of the ventral part of the myotome with its strongly developed outer layer (*o.f.*). The vertebral fibers mentioned above are not visible, but their position in a few following sections is indicated by a line one can imagine to be drawn between the points marked with the figure ⊕. It has been considered unnecessary to figure the myofibrils longitudinally under large magnification. The cross-striation is very plainly visible and is the same in all parts. Each of several regions of this embryo was examined and in all its parts were the same conditions found. We may sum up by stating that the embryo of 9 days age shows no electric tissue and but a very weak indication of the development of such a tissue, all of the myotome cells being decidedly of the muscular type. Our only evidence is drawn from future stages, as to the position of such electric tissues and from the otherwise unexplained precocity of the fibers in our 9-day embryo, in that same position.

STUDIES OF AN EMBRYO TWELVE DAYS OF AGE.

This animal (text-fig. 6) showed a considerable increase in size, not so much in length as in thickness. The posterior part of the body of this specimen was divided into the following pieces: First, from a short distance behind the anus to 14 vertebral segments further caudad. Two-and-one-half segments were cut off of this by serial, transverse sections from the posterior end (region $A - 1$) and the remainder was cut into vertical, longitudinal sections from right to left (region *A*). Second, 15 segments more were removed and 3 segments of the posterior end were again cut as serial, transverse sections (region B_1) and the remaining 12 segments were cut serially in horizontal, longitudinal sections (region *B*). Third, 14 segments were again cut off and the posterior 3 segments were cut transversely (region C_1), while the remainder was cut in vertical, longitudinal sections (region *C*). The next 18 vertebral segments formed a piece that was cut in vertical, longitudinal sections (region *D*), without any transverse sections being made, and this left a small bit of somewhat curled tail-tip, which was so young in development that its segments could not be easily counted. This is cut transversely in series (region *E*).

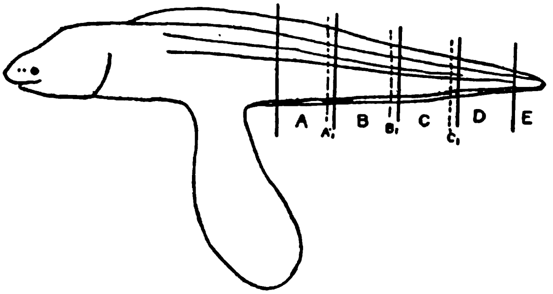

FIG. 6.—Diagram of body of an embryo of *Gymnarchus* 12 days old. Lines and letters indicate the regions studied. For explanations see text. (Copied from the same source as fig. 5.) × about 3.25.

Study of this embryo may best begin by examining a transverse section through the posterior part of the body (fig. 5, plate 2) to see what has become of the inner parts of the myotomes. Looking first for the point *L.M.* as seen before in figure 1, plate 1 (marked with ○), we can see at once that it is present as a compact mass of muscle-like tissue now clearly separated from the rest of the myotome. Further, there are to be seen on each side three other similar sections of the same muscle-like tissue, all more or less also separated from the main mass of the myotome. The most important part of our study now consists on the one hand in proving that this mass *L.M.*, in figure 5, plate 2, is derived from the undoubted muscle-fibers (marked with O), as seen in figure 1, plate 1, and on the other hand in showing that this same structure is to become the finished electric tissue as seen in such advanced development as in figure 21, plate 8, for instance.

In tracing it back to the muscle, we are much assisted by the fact that the dorsal and ventral spindles are always in an earlier or in a less complete stage of development than the upper middle spindle, or, particularly, the lower middle spindle under consideration. And, since in this embryo of 12 days the development has gone to considerable length, and since a slightly younger stage, say a 10- or 11-day embryo, was not included among the embryos at my disposal, this fact is of much importance, because it will be fair to take the left ventral spindle as an intermediate step in the comparison. Figure 9, plate 3, is a highly magnified section of the locality of the left ventral spindle from region *C* in the embryo of 12 days, and in it we can see a mass of muscle-like cells, closely associated and lying at the inner edge of the myotome. Certain changes clearly differentiate them from the rest of the muscle, however. One change is the fact that the myofibrils have shown a large diminution in size or thickness and have also suffered in power to take the stain; particularly on the periphery of some of the muscle-columns they almost refuse to take it. Their spacing is also irregular and they show a distinct tendency to clumping together and, in some cells, to get close to the nucleus or even to surround it. They can be readily compared in figure 9, plate 3, with the well-developed young muscle cells just outside and to the left of them. The dotted line marked *XX* indicates a separation of the two. All of those to the right of this line show the condition of the myofibrils mentioned above; those to the left show the usual condition of muscle cells of this age in fishes.

A second characteristic of the changing muscle cells under discussion is in their cytoplasm. It appears more abundant, although this may be due to the smaller fibril bundles. But it also stains more heavily with such stains as eosin, erythrosin, and orange G. With the eosin, for example, it also shows a more yellowish tinge than the cytoplasm of the usual muscle cells in the same sections. And lastly, some of the muscle cells seem to have entirely disappeared or to have greatly shrunken. This latter fact causes a loose and separated condition to obtain among the metamorphosing cells which shows in sharp contrast (fig. 9, plate 3) to the compact condition seen in the typical muscle cells to the left of or outside of the line *XX*.

Another important fact can be seen among the changing cells in figure 9. Those near the center of the group show a tendency to touch or coalesce with each other. Already at this early stage this marks a difference in the group. Those cells within the dotted circle are destined to unite with one another in a compact bundle to form the future electroplax; while those of the group which lie outside of the dotted ring are to degenerate and atrophy altogether, in order to make room for the growing electroplax. Just mediad of, or to the right of, the dotted ring is seen a cell that has entirely lost its myofibrils and whose cytoplasm is vacuolated and about to be absorbed. At other points, to the left, still other cells can be seen that have dwindled to a smaller size than any of the normal muscle cells. The cells within the ring are judged by the writer to be those which will form the electroplax at this point, because they are starting to unite and, also, because they occupy the position at which the electroplax will lie. Another reason is that their myofibrils are weakening and clumping. Some of those cells lying inside of the ring may also atrophy. This can not be infallibly judged; but certainly all of those outside of it and to the right of the line XX are about to degenerate and are not found in later stages.

The connective tissue creeps into the neighborhood of and among these metamorphosing muscle cells at this time, and good mitotic figures can be seen, showing that it is increasing the number of its cells. It does not, however, penetrate the groups of future electric cells inside the dotted line. Also blood, pigment, etc., are to be seen in characteristic positions.

A transverse section of the body at region B_1 need not be illustrated at this point by a low magnification figure, because it is so like figure 5, plate 2, in general appearance. But it happens that in such a section several very interesting stages, forming a sequence of which figure 9, plate 3, can be taken as the first member, were noticed, and figure 10, plate 3, is the second in this series. This drawing represents the right ventral spindle in region B_1, and a number, five to be exact, of the transforming cells can be seen here in closer union than the corresponding cells were in figure 9, plate 3. One or possibly two of these may disintegrate a little later. A dotted ring is not necessary, because the connective-tissue cells have partly marked off the electric cells, and outside of this incomplete ring and above it can be seen two muscle cells which are atrophying. A third cell is shown in a final stage of disintegration. Its cytoplasm is almost clear, or all gone, and its myofibrils have united in a single lump, which will soon become a round droplet or cell-inclusion that will afterwards disappear.

Moving to the right upper middle spindle (fig. 11, plate 3), we need very little explanation to see how the four or more muscle cells that first composed this structure have come into a still closer union. Below and to the right (outer) are still seen some of the degenerating muscle cells—five in this figure.

Several important points must be discussed in connection with this figure; the component cells, as seen here, are not simple, single muscle fibers. The lower one can easily be seen to have two myofibril bundles as well as two nuclei. The presence of two widely separated fibril bundles, as well as the large size of the cell, makes it nearly certain that the structure was formed by the coalescence of two muscle fibers. In the upper region of the future electroplax, however, a large cell is seen which has only one fibril bundle and four nuclei. It is possible here that this was one cell and that it is growing in size and multiplying its nuclei by amitotic division. As this is the same process which goes on in ordinary muscle cells of this age, it is not surprising to find it going on here, and in older specimens we shall find it the rule. The cytoplasm of all these electric cells is abundant at this stage and is dense staining with the acid dyes.

As compared with figure 11, plate 3, the next illustration, figure 7, plate 2, is most interesting and is a step of some magnitude in the development of the electroplax. The cytoplasm of such cells as compose this young electroplax is all united into a single mass and the relation of nuclei to fibril bundles is completed. The nuclei are always peripheral and the fibril bundles appear to form a single central mass. This is the permanent condition which will obtain throughout the life of the organ, and is also the condition common to some other electroplaxes, as, for instance, *Raja* and *Mormyrus*.

Just how the several fibril bundles become massed as a single bundle is not to be positively stated at this time. The individual bundles can hardly be imagined as moving together through the cytoplasm. It is probable that some of the several bundles as seen in figure 11, plate 3, are lost and absorbed, but it is not probable that all but one are so removed. The central bundle in figure 7, plate 2, looks large enough to be composed of several, such as are seen in figure 11, plate 3. The best explanation is that several remaining bundles are moved toward each other by growth currents in the cytoplasm, or by the absorption of material which lies between them. At the same time, of course, the peripheral cytoplasm is growing in mass and all nuclei tend to remain in this external layer. We shall see later that a very few nuclei are left behind in the central fibrous mass in some electroplaxes.

In figure 7, plate 2, we see, plainly and indubitably, the first form of the electroplax as found in older fishes.

The connective tissues which surround the electroplax are becoming more decided in figure 7, plate 2. Also blood-vessels and pigment-cells are oftener seen as in this drawing. One muscle cell, with its myofibrils clotted into several irregular masses and its nucleus in an advanced stage of disintegration, is seen just between the lower end of the pigment-cell and the electroplax.

It will be well at this point to examine some of the longitudinal sections of these early stages, in order to make clear several points which can not be so well studied in the transverse views.

Figure 12, plate 4, is a low magnification picture (× 140) of 6 segments of the caudal part of the body at region *D* in this 12-day embryo. Only the dorsal part of the body is shown, where a fortunate slant of the section has permitted the knife to pass through both the dorsal and the upper median spindles at the same time. The drawing is an accurate projection from three different sections, so that all parts of each spindle might be shown, as well as their relations to the rest of the myotomes. Bracket No. 1 (zone 1) in this figure indicates the inner tips of the ventral halves of the myotomes. Zone 2 shows a mass of connective tissue and nerve which runs between these two halves (compare fig. 5, plate 2, *Conn T.*). Zone 3 embraces the inner tips of the dorsal myotomes where they lie between the upper median spindle and the horizontal connective-tissue septum. It will be noticed that this set of muscle fibers appears to be very short, much shorter than the connective-tissue regions between them (not filled out in this drawing). The reason can be seen at once when these same muscle cells are examined under the high power (fig. 15, plate 5, bracket 3; and fig. 6, plate 2), where it is apparent that they are degenerating fibers. Parts of four of these fibers are shown in figure 15, plate 5, and that one nearest the electric tissue which is represented in figure 15, plate 5 (brackets 4 and 4_1), is the farthest gone, having lost its myofibrils altogether. The nuclei are also distorted and, besides the single large nucleolus, are noticeably empty of any chromatic matter or even of linin (notice the nuclei of healthy muscle and electric tissue). The next fibers to the left in this figure serve well to show how the degeneration takes place from the ends, where the cytoplasm is gathered in heavy lumps which stain light and yellowish with eosin. In fact, there is a singular similarity between the process of degeneration of these muscle cells and the transformation of the others into the electric tissue. Above, in zone 3, is a rounded cell which I take to be a muscle cell in a final stage of dissolution. It contains large granules of a chromatic substance, which appear to be the remains of myofibrils. Figure 6, plate 2, also represents three fibers from this same region in another myotome and shows three stages of the degeneration of the muscle. Two of them indicate that the degeneration begins in the middle of the fiber.

Zones 4 and 5 in figure 15, plate 5, as well as in figure 12, plate 4, show the young electric tissue of the upper median spindle. Let us first consider this tissue in figure 12, plate 4, where we can get a larger, low-powered view of it. In the first place, the most noticeable feature of the electric tissue here is that it shows scarcely any signs of a division into the original myotomes. A trace of this is seen in zone 4, but in a careful and systematic search through a number of the spindles of this stage very few instances could be noted. Even the myofibril bundles of successive myotomes seem to have united, and these bundles were most carefully examined under a Zeiss 2 mm. 1.40 ap. lens. I have no doubt that each spindle does constitute a continuous reticulum of muscle cells at this period. Laterally, the various muscle cells are not continuously united, but, as is shown in figure 15, plate 5, they are united at a number of points much in the way that some heart-muscle fibers are.

The myofibrils are best seen, of course, in these longitudinal sections, and it can be seen that they are typical. The transverse striation is as perfect as in the functional muscle, but owing to the slighter fibrils they are not quite so dark. In the upper median spindle of figure 15, plate 5, some points were found where this striation was slightly weakened. Cases of relaxation and semi-contraction uniformly characteristic of the normal muscle prevailed. The distances between the bands, as well as the length of the anisotropic parts, was the same as in ordinary muscle and remains as long as striation can be seen. This same fact does not hold true in *Raja* or in *Astroscopus*, where it is much shortened. It does hold, however, in *Mormyrus*, which is closely related to *Gymnarchus*.

The longitudinal sections, shown in figure 12, plate 4, and figure 15, plate 5, correspond to the spindles before it has become possible to see that they have segmentally divided into electroplaxes and before a central core of fibrous material has become definitely differentiated from a superficial layer of cytoplasm containing all of, or nearly all of, the nuclei.

We will now advance toward the head of this same specimen, to find material in a more advanced stage of development, in order to study the segmentation of the embryonic spindle into its individual electroplaxes (this segmentation has just been described as absent in fig. 12, plate 4), and to study further the differentiation of the inner fibrous core from the outer layer, and lastly to see how the myofibrils lose their transverse striation (muscle striation). The reader will remember that at this stage the anterior electroplaxes are in advance, developmentally, of the posterior ones. Figure 13, plate 4, shows four vertebral segments, taken from the region *C* of this same embryo, under low magnification. Bracket 1 embraces the zone of the epithelium. No connective tissue is shown. Between the muscle-zones 2 and 4 lies the zone of the long, narrow, embryonic electric spindle, marked by bracket 3. It is the lower median spindle which is shown, one which is always in advance of all the others in development. In this figure it becomes quite plain that, while the muscle tissue is arranged segmentally to correspond to the vertebra, the electric organ is not. In this case the electric spindle is separated transversely by connective tissue into three segments, instead of four. Nor is this proportion always maintained among the several electric spindles themselves.

In some cases, as will be seen later, a single electroplax corresponds to as many as five vertebræ. Even the electroplaxes do not correspond with each other. A subsequent figure will also demonstrate this (fig. 23, plate 9). Just what factors do determine the length of the electroplax I am unable to say. A closer study of the nerve distribution and blood-supply may throw some light on the matter.

The transverse sections of the stages represented in figure 13, plate 4, do not show anything of interest over and above what was discussed in connection with the conditions seen in figures 9, 10, and 11, plate 3, and figure 7, plate 2. We will, therefore, advance toward the head one step further in this embryo and examine a longitudinal section from the region marked *B* in text-figure 6. Here (fig. 17, plate 6) the electric tissue is seen in what may be considered as its maximum development in this 12-day embryo. The electroplaxes are distinct from each other and have grown considerably in size. The one selected for illustration is from a lower median spindle and shows an actual length of 0.9 mm. It appears with smooth, well-defined boundaries and good separation of core and outer layer. The myofibrils are shown in the core as several closely associated bundles, only to be distinguished from one another by a slight curving and twisting in their course. This would not be visible in a transverse section at most levels in the electroplax, and it gives strength to the view, expressed before, that the fibril bundles of a number of young muscle cells combine to form the single fibrous core of each electroplax. At the levels examined it would appear that a group of from 4 to 7 cells from each myotome is concerned in the formation of any electroplax and that these same groups of from 2 or 3 myotomes are likewise united end to end, thus making it possible that from 12 to 21 muscle cells are united to form each electroplax.

The beginning of the loss of transverse striation is quite visible in figure 17, plate 6. This striation merely fades or loses its staining power, beginning at several points, but usually at the middle of the electroplax. The ends retain the staining power of the *M* stripes longest.

The multiplication of nuclei must be spoken of at this point. It is well known that the nuclei of future muscle tissue divide by mitosis with a consequent and subsequent division of the cell-body as long as the cells are in the young myoblast stage. As soon as muscle differentiation begins to take place, the mitotic division of nuclei is changed to an amitotic type of division and the cell-body ceases to divide and begins to lay down myofibrils. Some few observations against this view are recorded, but it seems to hold in *Gymnarchus*.

In figure 15, plate 5, one can readily see many cases of amitotic nucleus division. The few cases of mitosis visible in the figure are of connective-tissue cells. Such cells, it is known, always divide by mitosis during their whole existence. As the electroplax grows, more nuclei are needed, and they are supplied by the amitotic divisions above mentioned. Just how long this process keeps up is not known, but it is probably mostly done before the first 15 or 20 days of development are passed. It is evidently going on fast in the 12-day embryo, and it is possibly finished in the 42-day stage. The nuclei are typical muscle nuclei in appearance and are not to be distinguished from the nuclei of real and active muscle in the same preparations, except, perhaps, they are slightly larger and have a somewhat heavier chromatic content (larger nucleolus and chromatic granules). This does not hold, of course, for the degenerating nuclei, whose differences have already been described. Connective-tissue nuclei can at once be distinguished by their delicate outline and small chromatin content, which is distributed as very small granules.

STUDIES OF A LARVA FORTY-TWO DAYS OLD.

In order to continue tracing this history of the development and growth of the electric tissue, we will now be obliged to pass to an embryo of some considerable size and one in which the oldest tissues are almost the same, for an understanding of the adult structure as those of a fully grown fish would be. Text-figure 7 gives an outline of this specimen, which was about 63 mm. long and whose tail part was cut off and divided and sectioned as follows: Beginning at about the level of the anus, a portion composed of 6 vertebral segments was sectioned in a vertical and longitudinal direction (region A); then passing caudad, the next 2 segments were cut transversely (region A_1); then the next 12 were cut longitudinally and horizontally (region C); then the next 6 were cut transversely (region C_1); then 13 others were cut longitudinally and vertically (region D); then 7 more were cut transversely (region D_1); then the next 14 were cut longitudinally and approximately vertically (region E), while the tip of the tail, composed of some 12 or more segments, was sectioned transversely (region E_1).

In the embryo of 12 days the youngest developmental stages of the electric tissue were found in the most distal portion of the tail, which at that time had but recently been extended by growth from the body and was still in process of extension. The oldest and consequently the most-developed electroplaxes were to be found in the anterior or cephalic end of the spindles.

In the present embryo, or larva, of 42 days, the conditions are reversed, and the electroplaxes in the extremity of the tail have passed those farther up in the body in their differentiation, and have reached a much greater and more complete development. Accordingly we will select for study one of the most anterior and least developed examples and compare it with that electroplax last studied, which is represented by figure 17, plate 6.

Figure 18, plate 6, is drawn from one of the ventral electroplaxes of the region C, as shown in text-figure 7. This position makes it fairly well forward in the spindle, although portion B would have shown slightly younger stages.

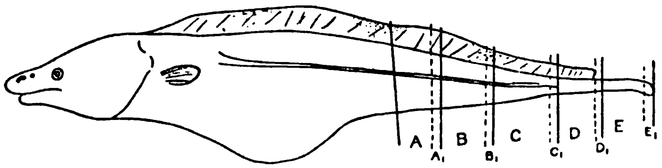

FIG. 7.—Diagram of body of an embryo, or larva, of *Gymnarchus*, about 42 days old. Lines and letters indicate regions studied. For explanations see text. (Copied from the same source as text-fig. 5.) × about 1 and 1.5.

The fibril core will first attract our attention. The first noticeable feature is that this core is growing in mass and volume all through the electroplax, but also far faster in its center than at either end. Throughout its course it has assumed a unified appearance which shows no trace of the several fibril bundles which have gone to compose it. In the narrower ends the mass is straightest and its component fibers appear most parallel, being but slightly wavy. As we follow them from either end towards the middle it can be seen that their course becomes more and more wave-like, until, in the middle, they have been thrown into decided folds. Their actual course can be somewhat better followed with thicker sections and deep eosin staining than with preparations of the usual kind.

As to the fibrils themselves, they have lost the transverse striation altogether. By this I mean that it is no longer stainable. It seems that, in the straight ends, with a good immersion lens one can see traces of this striation, left as a thickening of the fibrils, at points that may represent the previous position of the anisotropic *M* spindles.

Whether the increase in the mass of the fibrillar core, which goes on as the electroplax grows, is due to the thickening of the individual fibrils or to the laying down of more and new fibrils or to the deposit of an interfibrillar substance between them, was not decided. The first two conditions seemed the most probable, because of the apparent absence of much interfibrillar substance in the oldest and largest electroplaxes.

The cytoplasm of the outer layer begins to be of interest at this stage. Its most particular point of interest lies in the fact that it is decidedly different in structure at its anterior end from its posterior end. This is shown in its staining capacity as well as in its actual structure. In the specimens stained in iron hæmatoxylin and eosin the cytoplasm at the posterior end stains deeper than that at the anterior end, with both dyes. It can also be seen to be granular in structure—a sort of general granulation with a few larger granules of a substance which stains somewhat like chromatin. In particular, its cytoplasm is darker than the fibrillar core at this posterior end.

As the cytoplasmic layer is examined in an anterior direction, it is seen to stain lighter and at the same time to contain an occasional vacuole. This condition increases until, at the anterior end, about one-fifth of the length of the entire structure is covered with a cytoplasm which is so much vacuolated that it appears as a delicate reticulum in the meshes of which the nuclei lie. The fibril core extends out to the end or almost to the end, and it can be seen that it is darker in color and denser than the cytoplasm covering it. Some of the finer, granular material is found out as far as the reticulated tip.

A new element of interest begins to become apparent in this stage, and that is the point of attachment of the nerve. The strong medullated fibers come from the cord, pass through the spinal ganglion, and enter the connective-tissue "tube" of the spindle at the level of the posterior third of each electroplax. The fibers wind and turn considerably, in this vicinity, and are finally applied to the surface of the electroplax over an area which may be described in this specimen as its second sixth part from the posterior end. Thus the extreme posterior end does not receive any nerve-endings at this period.

The cytoplasm shows a number of indentations where the nerve is applied and the axis-cylinders of the nerve can be traced into these spaces, which they apparently fill with a club-shaped nerve-ending. This ending can not be satisfactorily described until some of the special nerve methods can be used to elucidate it.

Another and more simple step in development is indicated in figure 19, plate 6, which was taken from an electroplax in a dorsal spindle of this embryo at the region *E* (see text-fig. 7).

Two points of interest will be spoken of in connection with this stage. The shape has changed as follows: The middle part has both actually and comparatively widened over the breadth shown by the middle part of the electroplax seen in figure 18, plate 6, and this increased width is due to a broadening of the fibrous core alone, the outer nucleated layer remaining the same.

Accompanying this widening is also an actual shortening of the structure. Thus, part of the increased bulk of the middle is due to an absorption of the two ends. Apparently the anterior end suffers the greater amount of absorption, for it is usually shorter and thinner than the posterior. In this connection we must remember the previous vacuolization of this anterior end as seen in figure 18, plate 6. It would appear that the vacuolization was part of an absorption, or rather of an atrophic process. Considerable traces of it remain in figure 19, plate 6, and it is also still noticeable that the anterior end does not stain deeply. The posterior end retains its length and vigor to a greater degree. It seems to become considerably narrowed, however.

The form of the electroplax also begins to show a marked change through the development of spurs, branches, or papillæ which begin to protrude from both its ends, particularly from its posterior end. Some indication of this was visible already in figure 18, plate 6, as small lumps or "shoulders" that marked the organ roughly into three parts—anterior, middle, and posterior thirds. These growing spurs begin to give the middle third a distinct truncate or cylindrical form. The nerve fibers also attach themselves to the sides of the growing papillæ, particularly to their bases. The papillæ show an inclination to grow out of the sides of the posterior third.

We will now pass to the last and oldest stage of the electroplax which can be found in the embryos which Budgett collected in Africa. This is found in the *E* region of the 42-day embryo or larva of *Gymnarchus* and is represented in longitudinal sections under a low magnification by figure 23, plate 9, which shows parts, or the whole, of about 11 electroplaxes lying in place in the ventral, lower middle, and upper middle electric spindles of this region. Figure 21, plate 8, also shows a transverse section just cephalad of this point, in the *D* region, where cross-sections of the 8 electric spindles reveal partial or complete transverse sections of 7 electroplaxes, but only the electric connective tissue which fills the tube at this point, in the eighth.

Figure 20, plate 7, represents a longitudinal section of one of the electroplaxes shown in figure 23, plate 9, and will serve as a basis for our last study of this series.

The form has continued to follow the development which was indicated in figure 19, plate 6. The total length of the structure has shortened somewhat more, while its width in the middle part has increased twofold. These statements are the result of averages of about 15 measurements. The papillæ on the posterior surface have increased in length and some of them have begun to rival the posterior portion of the organ in length. We have no suitable figures of the adult electroplax, but from Fritsch's (3) descriptions, and from the tendencies shown by these embryos, it would seem that, as Ewart describes in *Raja*, the original posterior portion of the electroplax shortens and the new papillæ lengthen until they all form approximately similar structures. This can only be completely studied when we have secured suitable sections of the grown fish.

The development of papillæ is noticeably weak on the anterior surface. The figure does not show as many as some of the electroplaxes in figure 23, plate 9, but it is a fair illustration. Neither does it show well the usual condition of the main anterior process of the cell at this time, which can be better seen, in some electroplaxes of figure 23, plate 9, to be still in evidence and of considerable length, but of very weak development. This anterior process shows no trace at this age of the general vacuolization of its cytoplasm which we saw in an earlier stage, and the fibrils extend as a very thin and uncertain core through its length.

The fibrillar mass which forms the core of the electroplax has now assumed what appears to be its permament condition. The fibrils are very fine and, after having passed straight down through the posterior process, are thrown into flat waves by the process of packing them into the shortening and widening middle part which now constitutes the principal bulk of the structure. This causes the larger part of the fibrils to lie nearly at right angles to the longitudinal axis of the organ, and this appearance was first taken by the writer, who examined the oldest embryo first, to be a trace of the transverse striation of muscle from which the organ is formed. We now know it to be the myofibrils, lying at right angles to their original course. No conclusion was arrived at, in this stage, concerning the growth of this mass, as to whether the number of fibrils was increased, or whether the larger size was due to a thickening of the original fibers, or to the deposit of interfibrillar substance. The fibrils seemed to be as fine if not finer than in the earlier stages; certainly they are much finer than functional myofibrils. Since muscle tissue increases the number of its myofibrils long past this age, I see but little reason why this modified muscle should not also do so.

A closer study of the cytoplasm and nuclei of the peripheral layer was next undertaken in this oldest stage. Beginning on the posterior surface and on the papillæ, we find the layer thickest here and composed of at least three distinguishable materials. One was a dense material which was reticular in structure and stained with chromatic stains deeper than almost any other pure cytoplasm that I know of. This material was one constituent, while the other substance composing the general field at this point was a far lighter staining material which was homogeneous and clear. This latter seemed to bear the same relation to the denser material that the "nuclear sap" does to a linin alveolum or reticulum in the nucleus. Like the linin reticulum, this denser material was more refractive and took more of the chromatic as well as more of the acid counterstains than the homogeneous material did. I shall adopt Schneider's (31) name of "Linom" for the denser substance and his name of "Hyalom" for the clearer and more homogeneous material. It seems probable from the works of Bütscheli (9), Rhumbler (27), Hardy (35), Wilson (34), Andrews (1), Strasburger (32), and others that these structures of cytoplasm, as seen under the microscope in fixed material, do not represent the exact condition as it exists in life. After reading the pages of my recent work on the electric-motor cells of *Torpedo*, one can more easily see that the cytoplasmic linom is a structure which depends upon the fixative used as one factor and upon the chemical and physical peculiarities and the contents of this plasma at the time that the fixative is applied, as another set of factors. Its reticular or alveolar arrangement can most certainly be immensely varied, and all these artificial conditions must be very much different from that which obtains in life. Since I have only a few fixed specimens to discuss, over whose earlier preparation I had no control, I shall not try to solve the question of what the structure was during life, but will merely describe the present specimen as it appears.

The linom of the cytoplasmic layer on the posterior end is very fine and can only be seen with the best lenses and under the best conditions. This holds particularly for the outer portion of the layer, for as we examine the inner portion the reticulum grows larger meshed until, at its point of contact with the fibrillar core, the meshes are quite easily seen.

The same is true as we examine this layer in a more anterior position. Here all the meshes are proportionately larger, until in the layer, as found covering the extreme anterior surface, the meshes of the linom are visible with ordinary high powers. I do not refer to the vacuoles which are found at various points, for there is a great difference between the largest meshes and the vacuoles, although the vacuole may be derived from, or originate in, an overgrown mesh. The meshes always contain the hyalom, while the vacuoles do not. My definition of a vacuole in this case will be a space in the linom into which the hyalom does not extend. Also, the rounded outline of a vacuole shows a surface tension between its content and the cytoplasm, which does not seem to exist between a mesh of the linom and its contained hyalom. These vacuoles appear to have contained a soluble substance during life, and the hyalom is not soluble in fluids used to prepare the specimen. Such vacuoles are found at various points in the cytoplasmic layer, most often at its outer edge, while the large meshes appear at the inner edge and around the nuclei. The vacuoles become so large and numerous at the anterior end of a stage such as figure 18, plate 6, that the cytoplasm looks like foam containing the nuclei and surrounding the unchanged fibrillar core.

In addition to this structural basis of linom and hyalom, another very prominent content of the cytoplasm is a series of granules of some material that is denser and somewhat more stainable than either of the others. These granules are very numerous and fine at the periphery, where they cause the outer part of the layer to stain deepest with eosin, and they lie in the finer-meshed linom, and grow larger and fewer as one examines the inner parts of the layer. They are of a slightly more refractive quality than the linom and stain deeper with chromatic dyes the larger they get. The largest also have some color of their own, a slight golden-brownish color, somewhat like that of pigment granules. While the smallest granules seem to lie in or attached to the strands of linom, the larger seem to lie in its meshes in the hyalom. They remind the observer of the granules found in certain other cells, particularly in the electric-motor cells of *Torpedo*, as well as in other nerve-cells. In figure 16, plate 5, these finest granules are seen in the outer part of the layer stained with eosin, while the inner part does not show the larger ones that lie there.

The larger granules are prone to gather about the nuclei and about the inner part of the electric layer, as seen in figure 22, plate 8, where they took the iron hæmatoxylin stain well. The larger, chromatic-staining granules are also found, in groups or singly, in many parts of the fibrillar core. While the larger ones found in the core, around the nuclei, and at the inner edge of the cytoplasmic layer seem to stand in sharp contrast to the more numerous and finer ones found in the outer part of the cytoplasm, one can trace steps between the two kinds.

The writer believes these granules to be the secreted or prepared nitrogenous material used by katabolic processes to produce electricity, either directly or indirectly. They must be, physiologically, the same granules described by Ballowitz (5) in the cytoplasmic layer of the electroplax of *Raja*, and by Schlichter (30) in the cytoplasmic regions on both surfaces of the electroplax of *Mormyrus*. In this latter they were very large and not easily demonstrated. The writer has seen some indication of them in *Astroscopus* (12), but they would seem to be noticeably absent in other forms, as *Torpedo* (although they have been figured here by Fritsch (20)), and in *Gymnotus* (3) and *Malopterurus* (6), where such granules as Ballowitz has described or figured would seem to be inadequate in size and number for so heavy a duty. It will be noted in the above list that the weak-electric fish seem to have these cytoplasmic granules best developed, while the strong-electric fish show least of them. It is possible that these latter possess them, or their equivalent, in a more fluid and less visible form.

The cytoplasmic layer is marked off from the fibrillar core by a very definite and sharp line. It can not be said that a definite membrane exists here, although one may exist. The boundary between the outer layer and the core is not an even or a straight one, but shows a wandering course, especially at the two ends. At some points strands of the linom seem to branch into the core (fig. 16, plate 5). At the anterior end in particular it shows many extensive and complicated invaginations of its granule-containing substance into the fibrillar core. Certain small portions of it also have been permanently left in the main body of the core, usually near its anterior end. These inclusions (fig. 20, plate 7) may or may not include the nuclei. They always contain some of the largest of the granules.

The nuclei do not show any internal peculiarities which would distinguish them as electric nuclei from some of the other tissue nuclei, particularly from muscle nuclei, which they much resemble. They have a large chromatic content and a particularly large plasmosome which stains deeply. They can be sharply distinguished from some other nuclei, as connective-tissue nuclei for instance, where the more delicate outline and different chromatic pattern is discernible at a glance.

Each nucleus shows some sign of a surrounding differentiated layer of cytoplasm. This consists of larger granules and a zone in which the hyloplasm seems to be in greater proportion than elsewhere. At different places in a preparation one may see more or less of a contraction zone around the nuclei. While this may be a physiological condition, it is more probably an artifact due to the fixing or hardening.

One interesting condition is to be seen in most of the few nuclei which become detached from the outer layer and included with some small portion of the outer cytoplasm in the fibrillar core. These nuclei probably become so placed during a very early stage, and the further they are separated from the layer to which they rightly belong, the larger they grow and the more diffuse their chromatin becomes. The plasmosome diminishes in size and the whole structure looks more like a connective-tissue nucleus, except that it is very much larger. I have seen this same condition in the electroplax of *Raja*.

It was not possible to find a real electrolemma or cell-membrane covering this electroplax. A connective-tissue covering, more or less closely applied to the surface, was always present, but the fact that this covering possessed its own nuclei seems proof that it was a real connective-tissue covering and not any product of the activity of the electroplax tissue. At such points, as this connective tissue did not actually touch the electroplax, a careful examination was made to see if some actual cell-membrane did exist. Beyond the fact that the outer edge of the electric layer was sharply defined and that its surface was rounded and even as if some membrane was present, no real membrane could be demonstrated, either by its refractive properties or by its color.

INNERVATION.

A general survey of the innervation is desirable, as too little exact topographical work has been done on those fishes in which the electric-motor centers are thinly distributed in character over large areas of the cord, as, for instance, in *Gymnotus*, *Raja*, and the mormyrids. Regions *D* and *E* were selected in the 42-day-old embryo as the most favorable parts to study. The work was not as exact as it could be if the investigator had had plenty of live material, especially adult material, on which to use some of the well-known neurological methods. But even in this embryo, which was well fixed in sublimate-acetic, much could be accurately made out, and it is hoped, too, that the description will prove suggestive.

The motor cells were first looked for in the spinal cord, especially that region which was posterior and in the neighborhood of the electric organs. Assuming for the present, as a law, that electric-motor cells are larger than muscle-motor cells which innervate an equal weight of muscle, it was very easy to find groups of large, heavy nerve-cells scattered through the substance of the spinal cord from near the anterior beginning of the spindles to the very last point in the tail to which they extend.

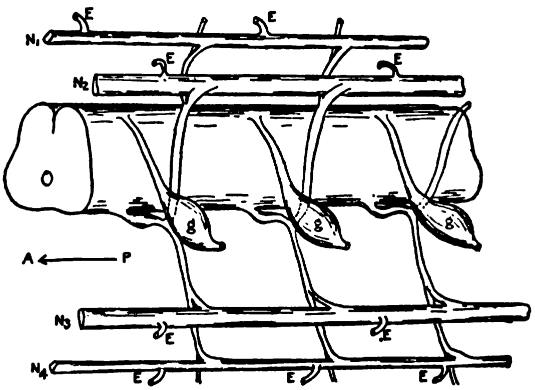

FIG. 8.—Side view of reconstruction (semidiagrammatic) of spinal cord and motor electric nerves of a larval *Gymnarchus* 42 days old. Arrow indicates anterior and posterior directions. N_1, N_2, N_3, and N_4 are the four electric nerves formed by branches from motor roots of spinal nerves. *E* indicates small branches from electric nerves that innervate posterior surfaces of electroplaxes. *g* marks spinal ganglion, whose afferent nerves have been cut off.

These cells were situated just above the central canal and from 1 to 4, or even 5, could be seen in every transverse section in most of this length (fig. 14, plate 4, *A*, *B*, and *C*). They did not appear to be divided into two symmetrical groups, but rather to lie in one median group. On the one hand, their dorsal position might appear to be evidence that they were not motor-cells or that they were not to be considered as modified muscle-motor cells. On the other hand, the presence of real ventral muscle-motor cells (fig. 14, plate 4, *D* and *E*) in the whole length of the cord, the correspondence of the cells under discussion with the position of the electric spindles, and the fact that Fritsch describes similarly placed cells in the same position in mormyrids as the motor-nerve cells of the electric organ, all seemed to constitute very strong indirect evidence that these were the nerve cells which innervated the posterior ends of the electroplaxes.

In addition, the writer was able to trace, in a number of cases, the course of the neuraxons through the cord, out into the ventral roots of the nerves, and finally into special bundles of nerve fibers that undoubtedly innervated the electric tissue. No one nerve process was actually traced for the whole distance unbroken, but the various parts and regions were so pieced together that the course was well established and corresponds in many ways with Fritsch's observations on *Mormyrus*. Text-figure 9 shows a semidiagrammatic cross-section of these courses as seen from in front, while text-figure 8 shows the same thing sketched in relief from the side. This topography will presently be described.

The electric-motor cells at this age must of course be considered as still very young, and descriptions from the adult are desirable. We will begin by examining the 9-day-old embryo once more to see if they have started. In this cord (fig. 8, plate 2) there are but very faint traces of any nerve-cell development, and some neuroblast mitosis is still going on. In the position to be later occupied by electric cells a little enlargement of nucleus and cell-body is visible. One cell here is of great interest, and that is one of the now well-known "Hinterzellen" or giant cells, first described by J. Beard (37), Rohon (29), Studnitzka (33), and others, in the embryos of *Salmo* and *Raja*, and later by Fritsch (21), as a different sort of cell, in the adult *Lophius*. Still later, such cells were described by the writer (36) in the embryos and adults of various Pleuronectidæ and in *Pterophryne*, where he showed it to be the same cell in both embryo and adult, and described the relations of anterior and posterior branches of the neuraxon. From its size, shape, and position in the present specimen, it seems that this dorsal cell might be, in some way, related to the electric-motor cells, but that question is easily settled when one examines the 12-day embryo and finds that all of the dorsal cells have effectually disappeared before the electric-motor cells begin to differentiate. Besides, the well-known fiber courses of the dorsal cells as worked out by Fritsch in *Lophius*, the writer in *Pterophryne*, Harrison in *Salmo*, and Johnson in *Catostomus* are sufficient proof that the two have nothing in common.

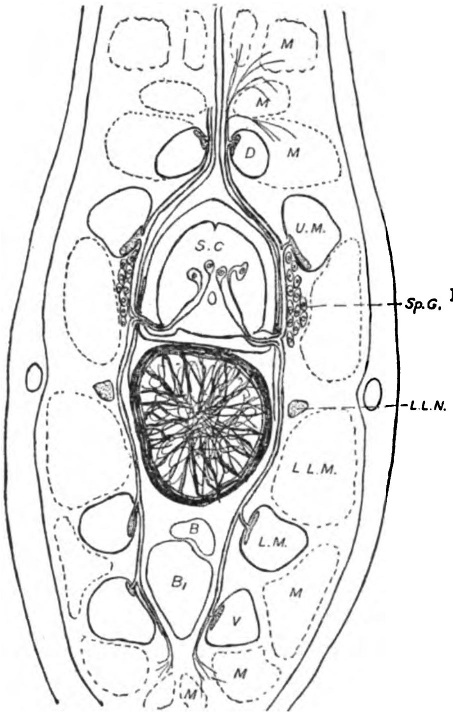

FIG. 9.—Diagram to show position and relations of nerve elements to electric spindles. *S.C.*, spinal cord, showing central canal and four motor electric nerve-cells. Processes from these cells pass out through ventral roots, and distribution of these roots to electric spindles and muscle-masses is indicated. *M*, some of the muscle-masses. *D*, *U.M.*, *L.M.*, and *V* show electric spindles on one side. *Sp.G.*, spinal ganglion; *L.L.N.*, lateral line nerve.

The 42-day larva shows the best development of these electric-motor-nerve cells until an older fish can be described. Here we find a heavy, rounded, cytoplasmic body of about 40 microns in diameter (fig. 14, plate 4, *A*, *B*, and *C*). Its outline is usually pear-shaped, with the axis-cylinder process given off at the pointed end. Dendrites are not visible in the specimen at hand. While most of these cells are of the same size, a few are noticeably smaller.

The nucleus is large, round, and placed nearly always in a very eccentric position. This position is most frequently on the side away from the neurite, but sometimes it may be very close to the neurite. Its diameter is about 16 to 19 microns in the largest cells of this age, and its outline is hard and round. The nucleolus consists of a single, or rarely double, plasmosome, which is a very little less than 5 microns in diameter, but which, when double or multiple, is of proportionally smaller size. As I have shown in the nucleus of the electric cells of several torpedoes, when a plasmosome is multiple its several parts, collectively, are larger in mass than is a single normal or usual plasmosome.

Of course, the nucleus was carefully examined as to any possible orientation of its nuclei, particularly the plasmosome, with reference to gravity or to the electric current or to the axis of the cell. Nothing of this sort could be found, although this does not preclude such a condition in the grown fish. It is known that in *Torpedo*, where such an orientation does exist, this same orientation is not found in the embryonic or larval stages.

From these cells thin, delicate axis-cylinder processes were traced down, as has been described, and into the ventral roots of the spinal nerves in a sufficient number of cases to assure the observer that they all ran in this direction. The process was thinnest shortly after leaving the cell, and became thicker as it approached the nerve-root. When it once entered the root it again became very thin, although now invested with a connective tissue and a medullary sheath. A large number of connective-tissue nuclei, nerve-sheath nuclei, and some unknown elements cause the motor-root of the nerve to swell to some size just after leaving the cord. It decreases again in size before it enters the foramen and leaves the vertebral canal in company with the much smaller dorsal root.

Just outside the canal the dorsal root ends in the spinal ganglion, and the motor-root traverses the inner side of the ganglion, from which it emerges on the lower edge, and at once divides into a dorsal and a ventral branch.

The ventral branch passes backward and downward (see text-figs. 8 and 9) to a point at a level with the lower middle spindle, and here it gives off a considerable group of fibers which pass caudad just inside of the spindle capsule. These fibers are joined by similar groups from others of the spinal nerves and the whole mass forms the lower middle electric nerve (text-fig. 8, N_3). At the posterior level of each electroplax this nerve gives off a few fibers (text-fig. 8, *E*) which branch out and innervate the posterior surface of this electroplax.

The remainder of the ventral branch passes farther down and again gives off a branch which goes to form part of the ventral electric nerve (text-fig. 8, N_4). This latter sends off little branches to furnish the posterior surfaces of the ventral electroplaxes with motor-fibers (text-fig. 8, *E*).

Going back to the anterior root, we find that its dorsal branch leads directly upward and, passing between the spinal ganglion and neural arch, slopes gently backward to give off a large branch which becomes a part of the upper median electric nerve (text-fig. 8, N_2). Its remaining fibers reach upward and furnish the dorsal spindle with a part of the fibers that form its dorsal electric nerve (text-fig. 8, N_1).

Of course, there are muscle-motor elements in both these branches of the anterior nerve-root, the number depending upon the position, backward or forward, at which we examine the arrangement. Two examples of the motor-nerve cells of the muscle-tissue from the cord in region *D* are shown in figure 14, plate 4, *D* and *E*. At the level of the anterior parts of the electric organ, when there are large quantities of muscle and the electroplaxes are very small, the muscle branches are large, particularly the dorsal branches, which have to supply the muscle-bundles for the large dorsal fin. In the posterior region of the tail, on the other hand, the muscle is almost entirely absent and the muscle-branches of the anterior roots are not even easily seen.

The eight (four on each side) longitudinal electric nerves are interesting in that they form a morphological buffer between the conflicting segmentation of the nervous, skeletal, and muscular systems, and the independent segmentation of the electric system. Were the electric segmentation to correspond with that of the others, we should not find these nerves in this recognizable form. In fact, in the anterior part of the electric organ we do not find them as continuous nerves, in many places, for more than two or three neuromeres at a time. And even posteriorly where they do form continuous nerves, the fibers that enter them from any given spinal nerve do not pass very far back in them before leaving to innervate one of the electroplaxes. Each nerve cell probably lies but a very short distance in front of the electroplax which it supplies. This was decided upon by plotting the relative positions of all motor-electric cells and all electroplaxes in the larger part of the organ.

While doing this it was also determined how many nerve-cells sent their nerve-processes to each electroplax. Thus, in region *E* of the 42-day-old larva there were easily counted 414 of the electric-motor cells, while in the same part there were 81 electroplaxes. This makes it quite sure that, on an average, about 5 of the nerve-cells were used for each electroplax. It was attempted to count the nerve-fibers as they left the electric nerve to branch out over the electroplax, but the elements were too small and the fixation not just what was needed to do this. It could be easily done in a grown specimen.

I shall now take up the structure of the nerve-fibers as they leave the electric nerves to pass to the electroplaxes, assuming that they have no peculiarities of interest during their course through the nerve-tracts between the cord and the electric chambers. (The electric chambers are not divided as in *Mormyrus* by a transverse septum of heavy, opaque, white connective tissue; this can be explained by the fact of their secondary segmentation.)

As the few fibers destined for any particular electroplax first leave the electric nerve they are directed caudad and are very small in diameter and invested with a fine connective-tissue sheath. This covering is probably medullated in the grown fish. They quickly turn in a gentle curve whose diameter is about half that of the spindle and pass forward through the electric connective tissue to the posterior surface of the electroplax. At the beginning of this curve, or just after leaving the electric nerve, the axis cylinder or neuraxon enlarges to a considerable diameter and becomes very wavy and irregular in diameter. Its course is no longer straight, and it is not possible to find a section of any considerable part of its length, except in some very few cases. Its sheath of connective tissue is loose fitting and the inner side shows a loose reticulum of fine fibrils and plates that form a weak connection between the axon and sheath.

It is at once apparent in the maze of fibers which approach the electroplax that the few neuraxones which first entered the compartment are now many in number. Still, it is difficult to see where they branch in the thin sections, owing to their sinuous and irregular courses and to the large numbers of transverse and oblique sections present. In several places this branching was seen, however, and recognized as the same multiple branching which has often been described in the terminal part of nerve-paths and, in particular, in the same comparative region of the electric tissue of *Raja* by Ballowitz (5) and Retzius (26), in *Torpedo* by several authors, and especially in *Malopterurus* by Ballowitz (4), who refers in this article to many other cases. The writer has also seen and figured it in the electric tissue of *Astroscopus* in a paper soon to appear. In the present case, one section, as can be seen in figure 16, plate 5, exhibits two cases of this branching, one of them showing a single fiber dividing into at least three or four branches; also, figure 25, plate 9, in which there is to be seen a well-defined single fiber dividing into two branches just before they end in two club-shaped nerve-endings in the electric layer of the posterior surface of an electroplax. The abundant nodes of Ranvier, described by Schlichter (30) in the case of *Mormyrus*, were not observable here, probably on account of the lack of osmic-acid fixation. I have no doubt that they are present and they must be present at the points at which the fibers divide.

As has already been stated, the fibers now approach and end on the posterior surface of the electroplax, as well as on the lower sides of some of the papillæ that arise from it. The mode of ending is not difficult to see, apparently, although this much studied and controverted question should not be approached lightly, especially where the material is merely a sublimate-acetic fixation stained with iron-hæmatoxylin and eosin.

The nerve-process, carrying its connective-tissue sheath until it actually reaches the surface of the electroplax, ends in a blunt and somewhat thickened knob which is embedded in a hollow or invagination in the substance of the posterior cytoplasmic or electric layer of that structure (see figs. 18 and 19, plate 6; also fig. 16, plate 5; also figs. 24 and 25, plate 9). This knob may be quite elongate in form and in some cases appears to be branching. The nerve fiber becomes very narrow and apparently dense just before entering the cavity, but it quickly broadens out, to as much as or more than its previous width, to fill the cytoplasmic cavity of the electroplax. Its substance becomes very light-staining, more so than any other part of its length is, and this light-staining quality is most apparent at its extreme distal end in the cavity. The nerve-fibrils, faintly visible in the outer courses of the axon and rather more so in the denser neck just before entering the cavity, can be seen in the light-colored club-shaped ending to be running in an irregular reticulum instead of in their previously almost straight and parallel manner. They could not be traced into the protoplasmic bridges between the club-shaped nerve-ending and the surface of the protoplasmic cavity in which it lies. In very few instances did the nerve-substance fill the recess in the electric layer tightly, probably owing to some shrinkage in both of the tissues. The small space between the two surfaces is crossed by the numerous fine processes or strands, mentioned above, of some of the nerve-tissue remnants, probably of an original closer contact.

The question now arises as to whether the cavity or depression which the nerve-ending occupies is to be considered as an invagination of the surface of the electric layer or as a real penetration of the electroplax by the nerve. I shall consider it as the former, because the surface of the electroplax appears not to be interrupted by the opening but to continuously follow the inner edge of the cavity all the way around.

The presence of a perceptible cell-membrane or electrolemma would assist in the solution of this question, but such an organ could not be demonstrated. The edge of the cytoplasm was sharp and definite, but no membrane was visible, either by its staining properties or by refrangibility. It possibly will be found in the adult organ. Nor was it possible to see the "Stäbchen" or rodlets which have been described in other electric organs by Ballowitz (3, 5) and his pupils (30). Some striated arrangement of the granular electrochondria or granules described previously was observable, but this constituted a fibrillar secretory striation, such as is seen in the surfaces of most cells that are undergoing exchanges of any kind. Here, again, we must await examinations of the adult electroplax before we can say if such "Stäbchen" are present; also if they are homologous with the well-defined rodlets found by Ballowitz in *Torpedo* and in *Raja*, and whether they are specific structures of any importance in the production of electricity.

Returning to the relation of nerve-ending to electroplax, we have practically decided that the nerve-club is embedded in an invagination of the surface rather than in a cavity in the substance of the electric layer.

In doing this it has formed a much more intimate connection than may be seen in other places where the nerve-fibers touch the electroplax very closely, even being partly embedded in it, or running through the fundus that lies between two papillæ, where the fiber is closely pressed on three sides by the surface of the electroplax. In these fiber contacts the connective-tissue sheath persists, while in the club-shaped or heavily rounded endings the connective-tissue sheath is lost, left at the surface, and the little protoplasmic bridges, shown when slight shrinkage has taken place, testify to the intimacy of the contact. In addition a slight amount of fine, golden-colored granules surrounds the nerve-ending, lying on its surface, between it and the substance of the electroplax (figs. 24 and 25, plate 9). These granules are not found on any other part of the nerve surface.

Naturally the paper of Schlichter (30) was carefully examined to see what he had found as to the ending of the electric nerves on the related form, *Mormyrus oxyrhynchus*. He had adult material, but otherwise was no better off than the writer in the possession of material which had been treated especially for neurological study. He describes the nerve-fibers with their medullary sheaths as coming in contact with the large process of the electroplax and then suddenly ending just as they reach certain large indentations of the surface of this electroplax. He found in these indentations only a little coagulated material and some nuclei.

The writer has no doubt that the slight coagulum represents what remains of a club-shaped nerve-ending similar to that which he finds in *Gymnarchus*. The nuclei, from their position in Schlichter's picture, are evidently the nuclei of terminal connective-tissue coverings. If this idea be correct we will have a very simple but interesting form of nerve-ending, much larger in size than that found on any muscle or any other electric organ and one in which it will be, apparently, easier to study the intimate contact of nerve-substance with motor-substance from a cytological point of view than in any other form. In particular, we should try to stain these endings with the nitrate of silver and methylene blue methods devised for neuro-cytological studies. This work, however, can be undertaken only on the ground, with good laboratory facilities and with an abundance of fresh material.

The writer has published observations on some peculiar horizontal, pointed rods, or pointed threads, found imbedded in the electric layer of the electroplax of *Astroscopus*. At that time he suggested that they might be in some way homologous with or related to the "Stäbchen" because of the absence of any other well-defined "Stäbchen" in this fish. Such structures are not found in the present *Gymnarchus* larva, but they have been seen and described in *Raja*, in a paper soon to be published. Their presence in *Raja*, in addition to the "Stäbchen," proves them to be entirely different cell organs.

One word in regard to certain possibilities for the physiological study of the electric organ in *Gymnarchus*. A recent paper by Bernstein and

Tschermak and another by Bethe go to show that the electric discharge of *Torpedo* and other fishes is produced by different concentrations of sodium chloride in the electroplaxes and in the intervening electric connective tissue. Since there is a long series, theoretically, of these alternate segments of higher and lower degrees of concentration of the electrolyte, and since all the higher concentrations are presumably equal, and all the lower concentrations are equal, we would have a series of equal potentials alternately opposed to each other, and the result would be zero or else only the strength of one concentration current, in case there was one more or one less of either of the concentrations.

To obviate this difficulty a membrane has been imagined, on one surface of each electroplax, presumably the electric or nerve-ending surface (posterior surface in this case), which will be permeable only to one kind of the ions, either negative ions or positive ions, and by which the current is thus rendered integral in one direction.

Two things remain to be proved in connection with the above theory—the fact of different concentrations and the presence of such a membrane. This can be done in any fish in which it is possible to effectively separate the segments in a fresh state, so as to submit them to delicate chemical tests. In *Gymnarchus* we have a fish whose elements are larger than those in any other one of the seven electric types—large enough to be cut apart, I believe, and analyzed separately in the chemical laboratory; also large enough to submit anterior and posterior surfaces to physical tests that may show its permeability to either positive or negative ions and its impermeability to the other kind in one direction. This experimental work can most certainly be done if the proportion between the bulk of electric connective tissue and electroplax remains the same in the adult as in the larva (see fig. 23, plate 9). Fritsch shows much less of the electric connective-tissue segments in his figures of the adult organ.

Numerous other anatomical features of *Gymnarchus* have caused it to be classed with the other mormyrid fishes. This fact makes it of interest to compare its electroplax with the very different electroplax in these fishes.

That found in *Mormyrus oxyrhynchus* will serve as a type and its general plan has been well shown by Ogneff (25) and Schlichter (30). Here it is evident that a number of consecutive and entire myotomes have been converted into electroplaxes and that the middle layer of each electroplax is composed of unaltered and clearly striated myofibril bundles. The large number of these fibril bundles, and their distribution, indicate that the whole electroplax in *Mormyrus* is a syncytium composed of all or most of the cells which would otherwise have gone to make up the single myotome. In this we find an agreement with the electroplax of *Gymnarchus* which is also formed from several cells. In the one case, however, all the cells in the myotome have been used (*Mormyrus*); in the latter only those lying in eight particular localities (*Gymnarchus*). (See paper by Dahlgren (36).)

Further homology is seen in the disposition of the probably superfluous myofibrils. In both forms they are relegated to a middle position in the electroplax, while the apparently more important cytoplasm forms layers on the anterior and posterior surfaces of the structure. Also, in both, the now useless myofibril bundles are packed out of the way at right angles to the axis of the electroplax, which remains the same as the former axis of the muscle-cells that were used to form it.

The only difference lies in the fact that the striation of the fibrils is retained in the *Mormyrus* forms, while it is lost in *Gymnarchus*, the dark-staining anisotropic substance apparently dissolving away.

From what little can be predicted concerning the possible origin of the electric tissue in the other teleost forms, it is probable that the Mormyridæ (including *Gymnarchus*) are the only fish in which the electroplax is formed as a syncytium from more than one cell. In *Astroscopus*, *Electrophorus* (formerly *Gymnotus*), and *Malopterurus* the structures show every evidence of having been developed from single myoblasts with the exception of *Malopterurus*, where it is a question as to whether they are not evolved from gland-cells instead.

SUMMARY.

In conclusion it may be stated that—

(1) The electric tissues of *Gymnarchus* are developed by the differentiation of certain portions of its normal, striated muscle-tissues during an embryonic or larval period extending from the ninth day to the forty-second day of embryonic life. The critical period of this change takes place in the neighborhood of the eleventh, twelfth, to fifteenth days.

(2) The muscle fibers which go to form the electric tissue give up their usual segmentation into myotomes, and first form eight long and continuous spindles which afterward segment, each into a lesser number of masses, the electroplaxes.

(3) The myofibrils lose their transverse striation and form a large inner core for each electroplax. They lie in a wavy mass, mostly at right angles to their former course. The active cytoplasm forms an outer layer which is denser and stains deeper on the posterior than on the anterior surfaces. It contains granules.

(4) Each electroplax is made up of several muscle cells, 12 to 20 or more. This is different from the two elasmobranch fishes, in which each muscle cell forms only one electroplax.

(5) The nerve is distributed on the posterior surface and ends in blunt knobs that lie in cavities formed by the invagination of the surface of the electric layer. The current probably runs from tail toward head, as in *Mormyrus*.

(6) The development of the tissue gives a strong clew to the probable development of the electric tissues in the other mormyrid fishes.

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EXPLANATIONS OF THE PLATES.

PLATE 1.

FIG. 1. Transverse section through body of 9-day-old embryo of *Gymnarchus* in region *C*. *L.M*, position of the large muscle cells that will eventually change into the lower median electric spindle; *V.F*, location of large vertebral fibers; *XXXX*, locations of electric spindles in an adult fish with reference to bony structures, blood-vessels, and spinal cord; *N*, canalis centralis; B_1 and B_2, caudal blood-vessels. × 110.

FIG. 2. Muscle-tissue in transverse section from 12-day-old embryo of *Gymnarchus* to show typical muscle cells. *A*, one of oldest fibers with two strong fibril bundles; *B*, growing muscle cell with fibrils being laid down in a ring; *C*, extreme upper edge of myotome with youngest cells at top. × 1150.

FIG. 3. Transverse section through group of larger muscle cells found at *L.M.* in fig. 1. Under greater magnification. For explanations see text. × 1150.

FIG. 4. Longitudinal vertical section of *C* region in 9-day-old embryo, ventral portion. Between marks ○ ○ are seen same parts of seven myotomes as are shown at *L.M.* in preceding figure in transverse section. Note heavy development of these cells. A line drawn between marks ⊕ ⊕ would indicate location of seven groups of vertebral fibers in next few mediad sections.

PLATE 2.

FIG. 5. Transverse section of body of 12-day-old *Gymnarchus* embryo in region CC_1. *D*, dorsal electric spindle; *U.M*, upper median spindle; *L.M*, lower median spindle; *V*, ventral spindle; *Conn.T*, connective-tissue septum; *N*, canalis centralis. B_1 and B_2, caudal blood-vessels. × 110.

FIG. 6. Three degenerating muscle cells from another (3) zone in fig. 12. × 1440.

FIG. 7. Electric spindle showing first stage where electric muscle cells can be called an electroplax. Complete coalesence has taken place. Nuclei are in peripheral layer. Myofibrils are concentrated in middle to form central fibrillar core of structure. × 1150.

FIG. 8. Transverse section of upper dorsal part of spinal cord in a *Gymnarchus*, embryo 9 days old. Neuroblasts, in region that will later show electric motor-cells, show some little differentiation and growth. Near, and above, this region is one of the transitory giant ganglion cells. × 940.

PLATE 3.

FIG. 9. Transverse section of left ventral spindle from region *C* in embryo of 12 days. To left of dotted line are normal muscle cells, to right are degenerating muscle cells, in the midst of which, and surrounded by dotted circle, are a group of muscle cells going through the first steps of transformation into electric tissue. × 1150.

FIG. 10. Transverse section of ventral electric spindle in process of formation in region *B*, of 12-day-old embryo of *Gymnarchus*. At left of figure two muscle cells begin to degenerate, while just to right of upper one of this pair a muscle cell is seen in advanced stage of atrophy. Connective-tissue capsule beginning to form. Electric muscle cells rather more coalesced than in fig. 9. × 1150.

FIG. 11. Transverse section of upper middle electric spindle in same section as fig. 10. Rapidly degenerating muscle cells to left. Further coalescence of electric muscle cells than in fig. 10. Myofibrils weakening but still in separate groups. × 1150.

PLATE 4.

FIG. 12. Longitudinal vertical section of dorsal part of region *D* of 12-day-old embryo of *Gymnarchus*, showing relations of muscle tissue and electric tissue. Bracket 1 indicates zone of degenerating muscle fibers on inner median edge of five myotomes, on ventral side of median connective tissue; zone 3 shows atrophying muscle fibers on inner median edge of myotomes, dorsad of median connective tissue; zone 4, rudiment of upper median electric spindle; zone 5, same of dorsal electric spindle; zone 6, muscle tissue from ventral spindle to dorsal outer edge; zone 7, connective tissue; zone 8, dorsal epithelium. × 140.

FIG. 13. Longitudinal vertical section of ventral part of region *C* of 12-day-old embryo of *Gymnarchus*. Zone 1 indicates ventral epithelium; zone 2, ventral outer portions of five myotomes; zone 3, lower median electric spindle which is dividing into three parts in five vertebral segments; zone 4, inner degenerating muscle fibers of five myotomes; zone 5, projected outline of vertebral segments. × 140.

FIG. 14. Motor nerve cells from spinal cord of 42-day-old larva of *Gymnarchus*. *A*, *B*, and *C*, three electric motor nerve cells. *D* and *E*, two muscle motor nerve cells. From region *E*. × 1500.

PLATE 5.

FIG. 15. More highly magnified part of fig. 12. Brackets indicate same zones. × 760.

FIG. 16. Highly magnified part of transverse section through an electroplax from *C* region of 42-day-old larva of *Gymnarchus*. Curving bundles of modified myofibrils are seen in core and cut at various angles. Outer or electric layer is fixed and stained to show finer outer granules or electrochondria. Vacuoles as well as larger meshes of the linom are visible on inner edge of electric layer. Electric nerves are cut in sections at many angles and show medullary or connective-tissue sheath and its nuclei. At two points branching of these nerve fibers is visible and at four points its contact with electric layer of electroplax is to be seen. × 1500.

PLATE 6.

FIG. 17. Longitudinal section of a young electroplax from *B* region of 12-day-old embryo. Segmentation of electric tissue is now complete into unit electroplaxes of which figure is good example. Central fibril bundle beginning to lose its transverse striation. Structure is as long as three muscle segments or bony segments, as near-by spinal processes show. This represents oldest stage in embryo of this age. × 210.

FIG. 18. Longitudinal section of young electroplax from *C* region of 42-day-old larva of *Gymnarchus*. Fibrils have lost all striation and become waved in middle part. Cytoplasm shows strong differentiation at two poles (ends). Anterior end strongly vacuolized. Papillæ begin to grow from ends of middle part and nerve-endings have become established at junction of posterior third with middle third. This shows youngest stage in 42-day-old larva and is a successively later step in differentiation than fig. 17. × 210.

FIG. 19. Longitudinal section of an electroplax from *E* region of 42-day-old larva of *Gymnarchus*. Fibral bundle much "waved" in middle third, which now begins to show form of electroplax. Papillæ more developed. Nucleus and some cytoplasm inclosed in fibrillar core. × 210.

PLATE 7.

FIG. 20. Longitudinal section of another electroplax from further caudad in *E* region of same larva of *Gymnarchus*. This represents oldest stage accessible and is regarded as almost adult in its histological characteristics. Two nuclei shown in fibrillar core; one of these shows characteristic swelling.

PLATE 8.

FIG. 21. Transverse section of body of 42-day-old embryo of *Gymnarchus* from *D* region. Greater part of muscle tissue is here transformed into electric tissue. Section passes through very last part of dorsal fin. Electroplaxes cut at different levels and in one case section passes through right ventral spindle showing only electric connective tissue. *D*, dorsal spindles; *U.M*, upper middle spindles; *L.M*, lower middle spindles; *V*, ventral spindles; B_1 and B_2, caudal blood-vessels; *N*, neural canal. N_1, N_2, N_3, and N_4, electric nerves; N_5, lateral line nerve. × 110.

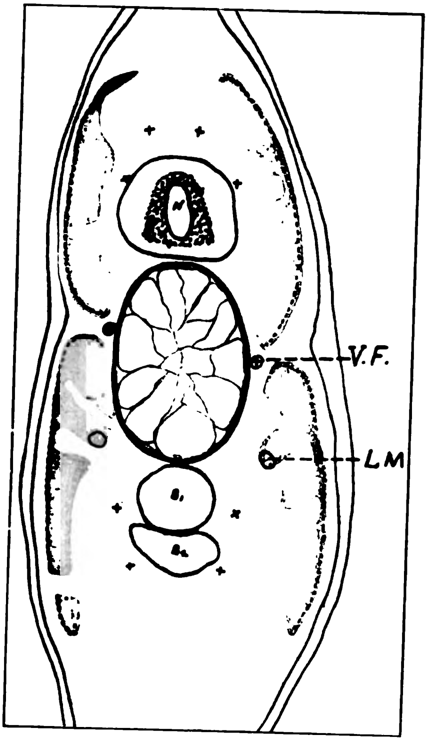
FIG. 22. Part of transverse section from middle part of an electroplax in *E* region of same larva of *Gymnarchus*. This figure serves well to show larger granules that lie deeper in electric layer, also connective-tissue sheath or wall of electric spindle. × 1500.

PLATE 9.

FIG. 23. Longitudinal section from *E* region, showing parts or whole of 11 electroplaxes lying in dorsal, upper middle, and lower middle spindles. Some remaining muscle fibers are visible at sides. Figure serves to show that there is no heavier, transverse connective-tissue wall lying in electric connective tissue, as is found in *Mormyrus*. This emphasizes secondary segmentation found in *Gymnarchus*. × 110.

FIG. 24. Small part from edge of basal part of a papilla on posterior surface of an electroplax from *C* region of 42-day-old *Gymnarchus*. Shows a single nerve, ending in a somewhat elongate and branched end-plate in electric layer. Outer crowded mass of granules stained deeply with eosin; larger inner granules not stained. × 1500.

FIG. 25. Small part of edge of another part of anterior surface of same electroplax. Shows a nerve fiber dividing and each branch ending in typical club-shaped nerve ending of *Gymnarchus*. × 2200.



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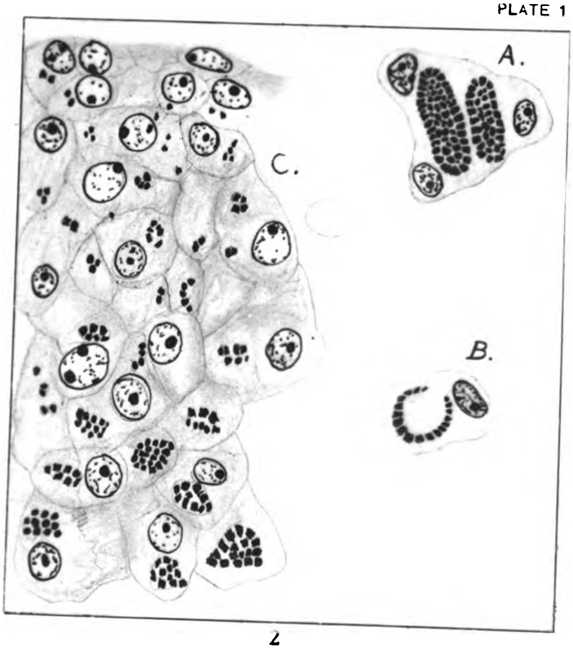

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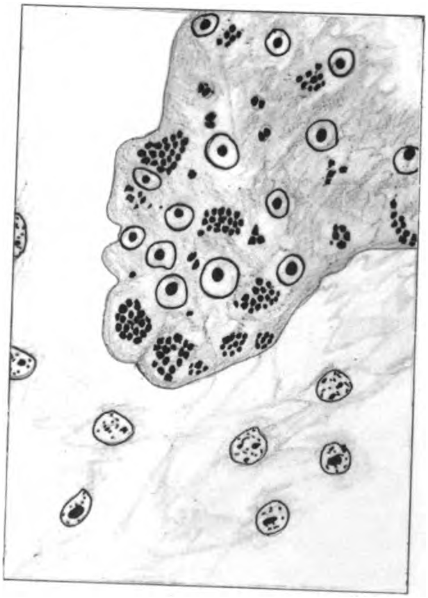

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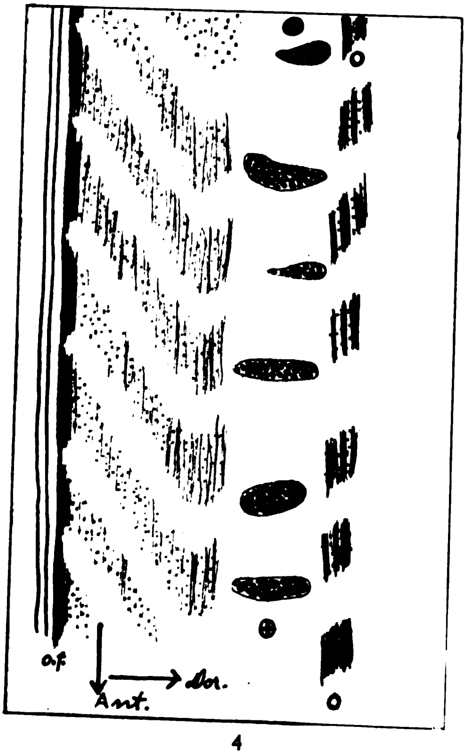

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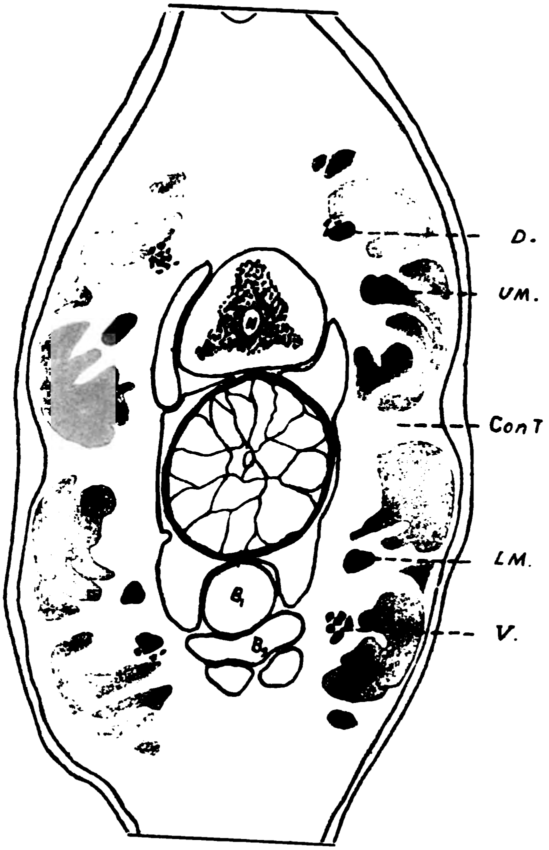

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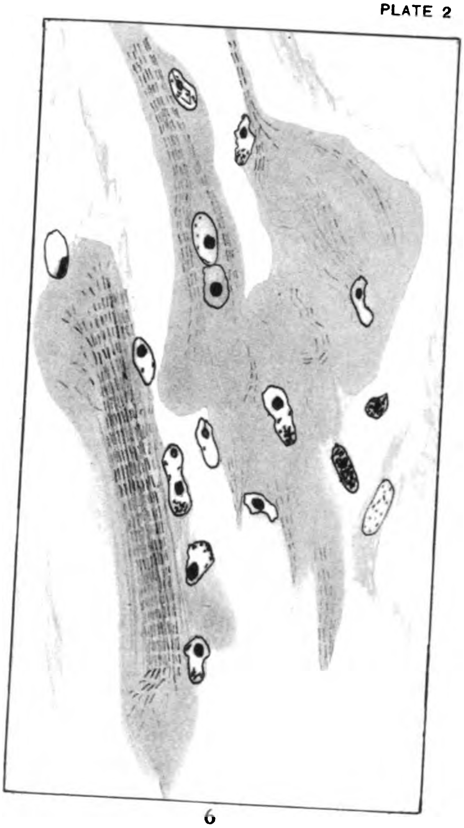

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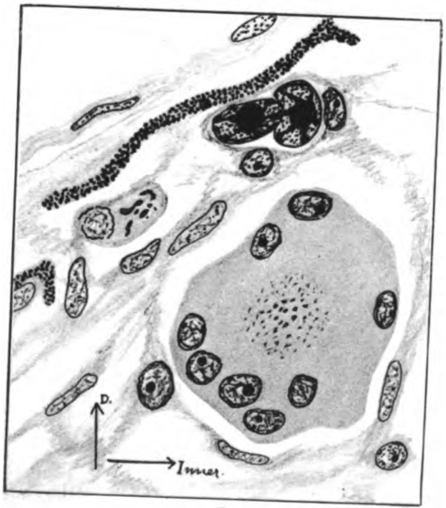

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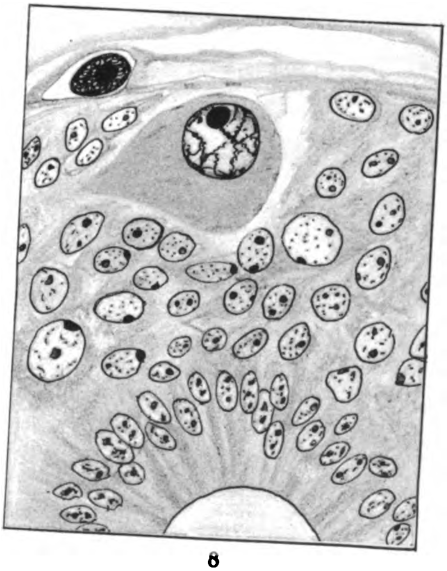

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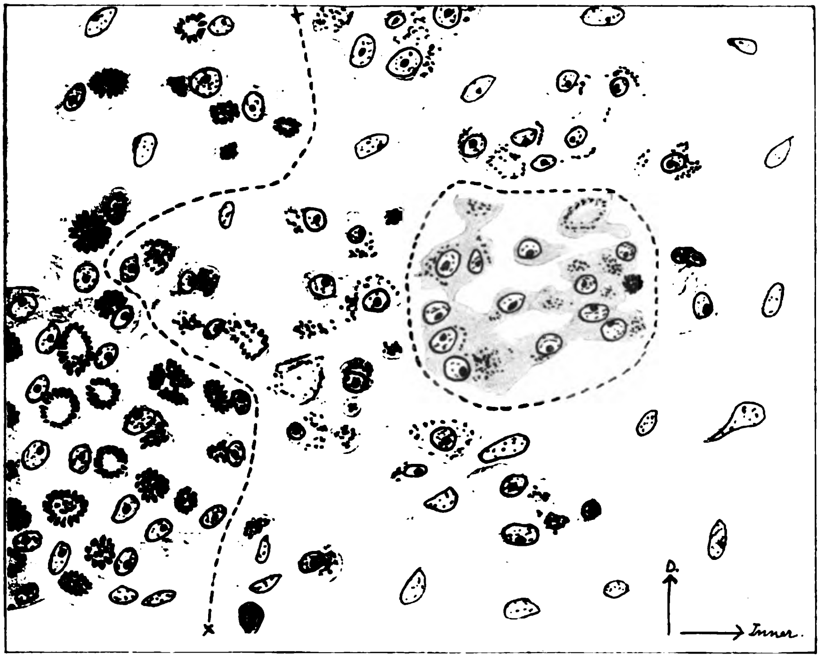

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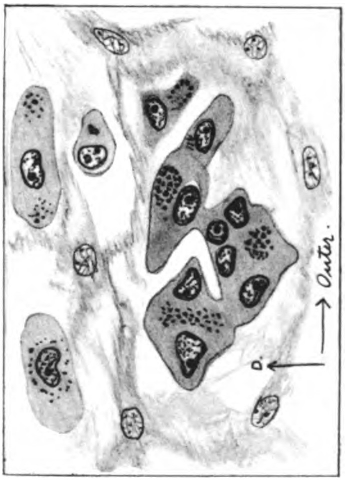

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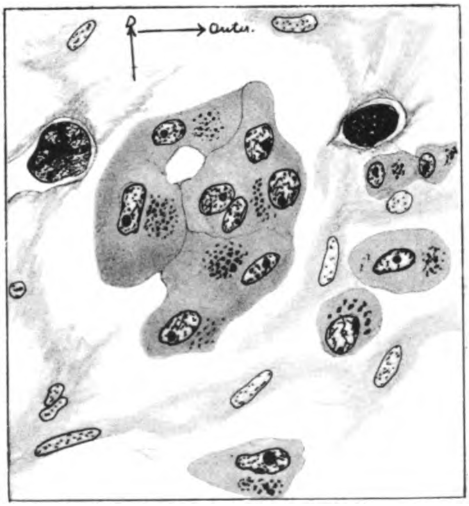

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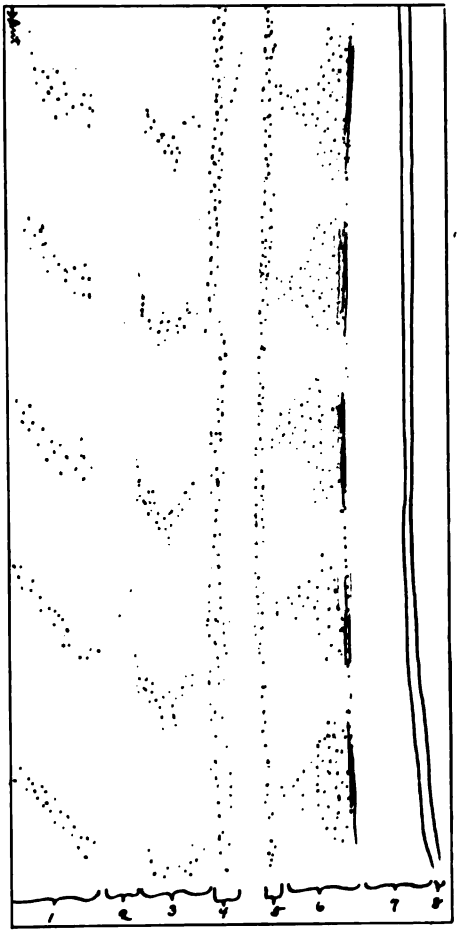

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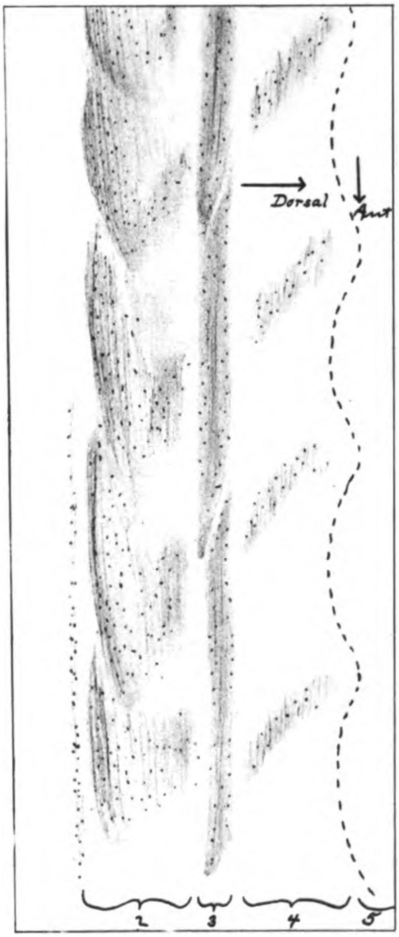

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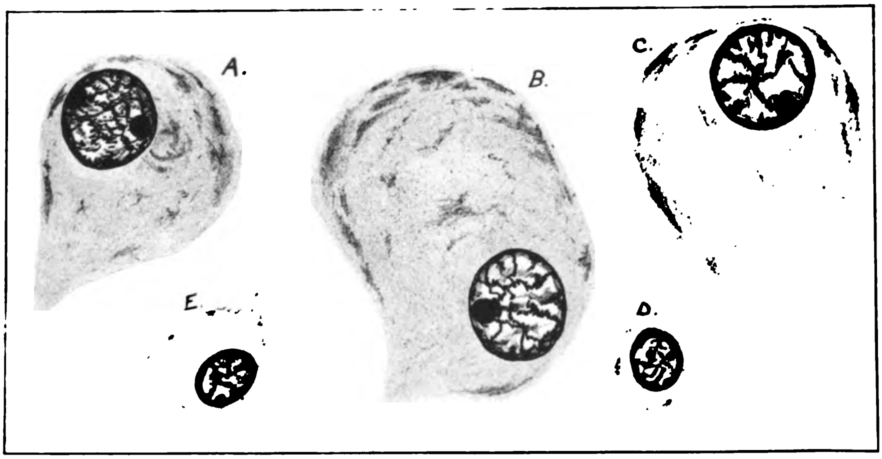

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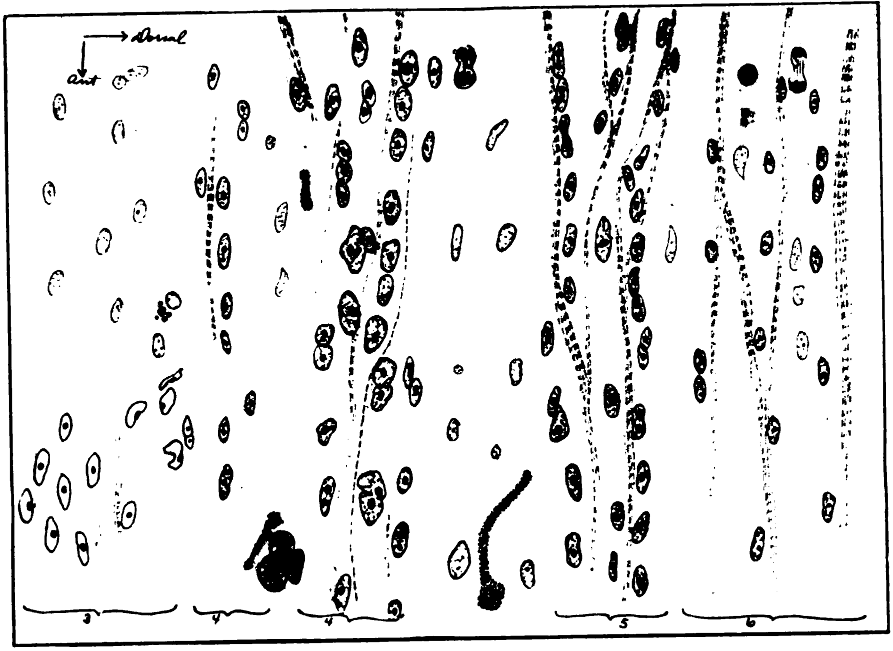

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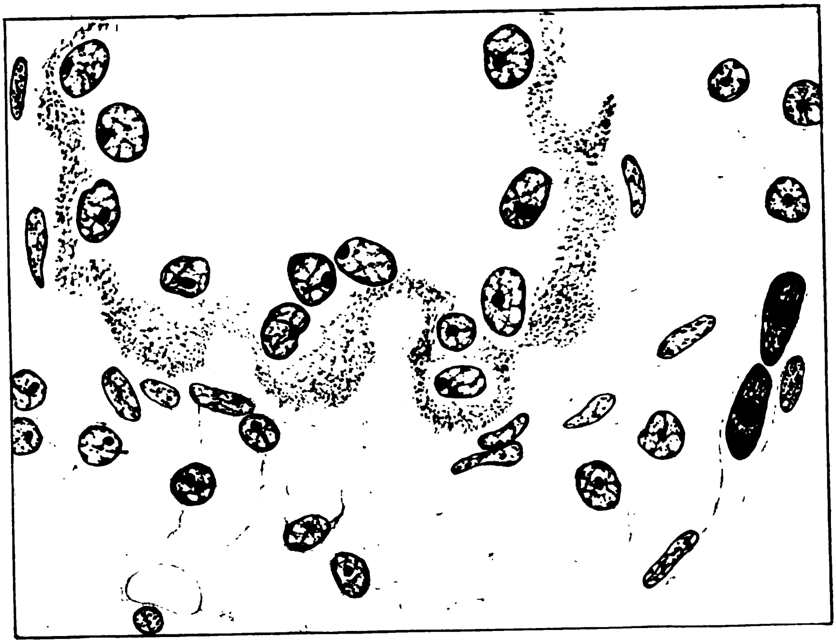

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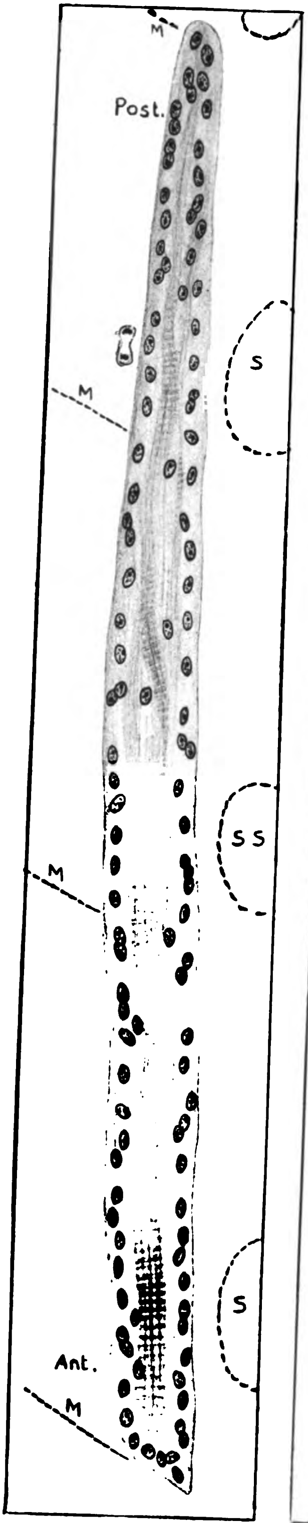

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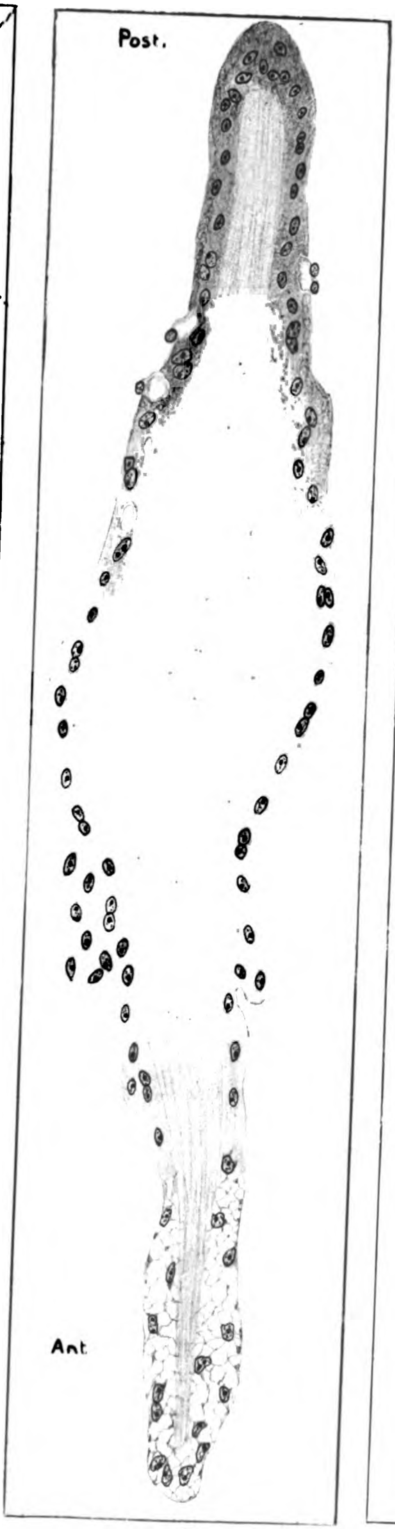

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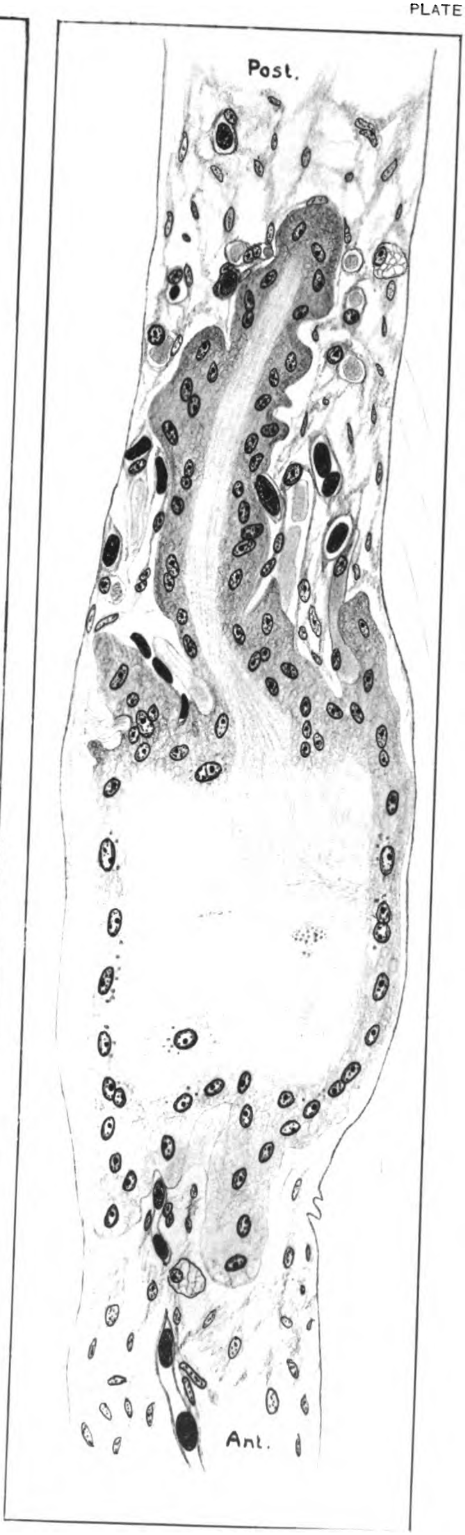

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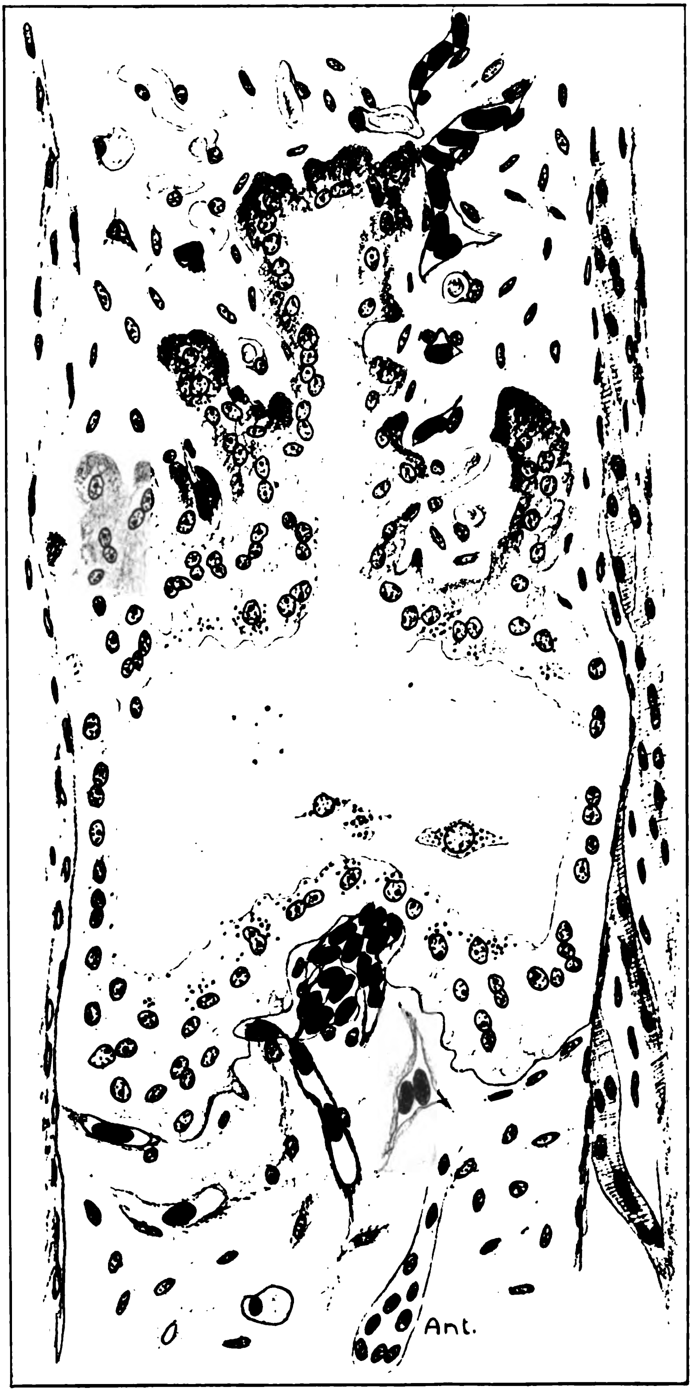
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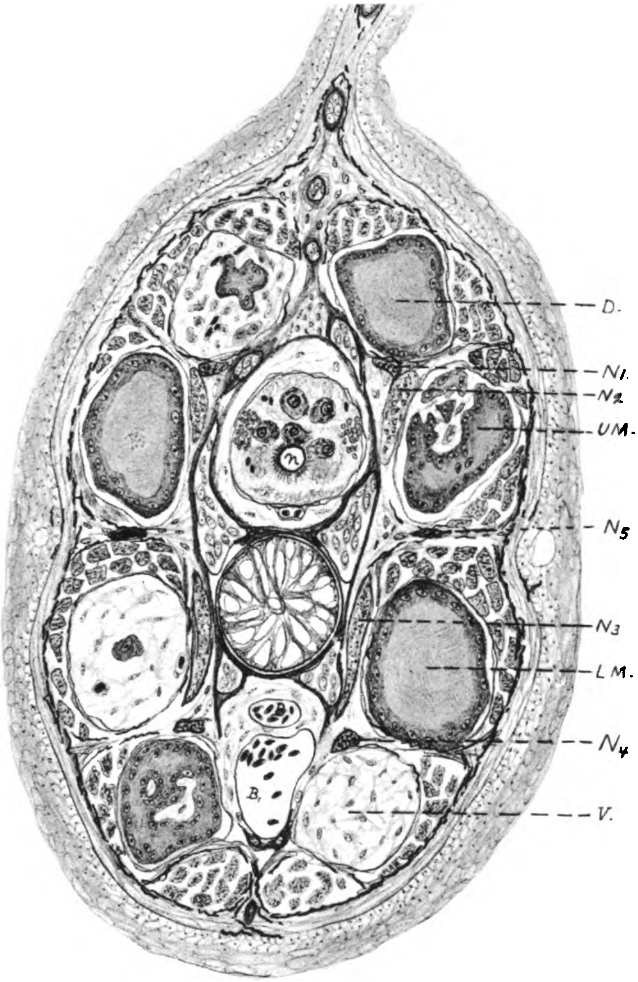

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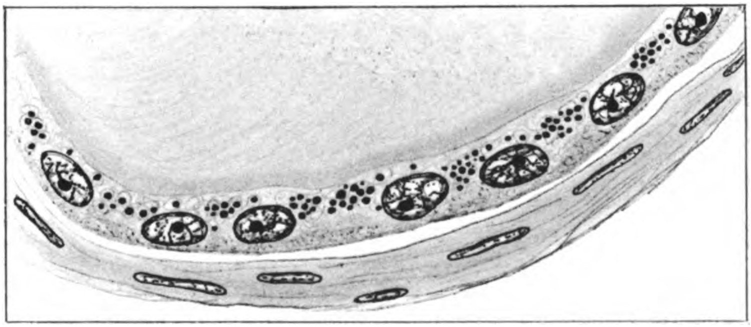

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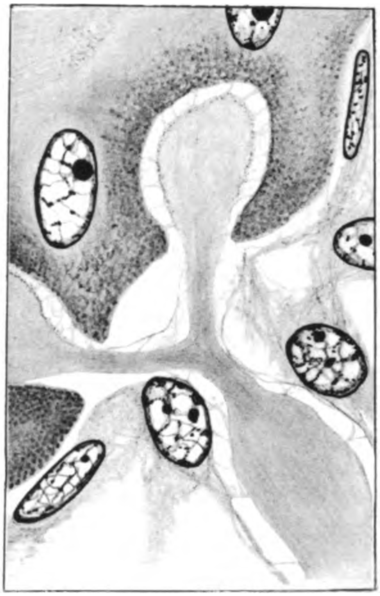

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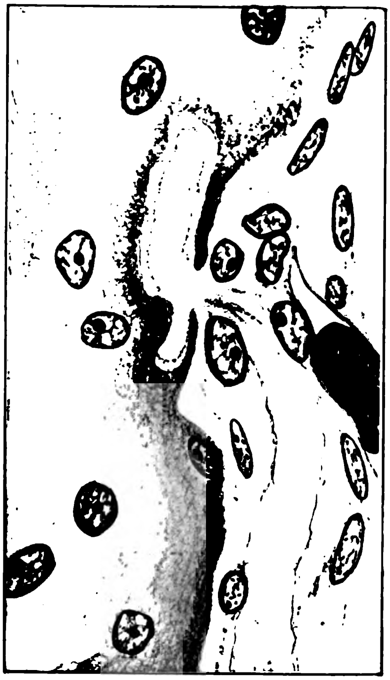

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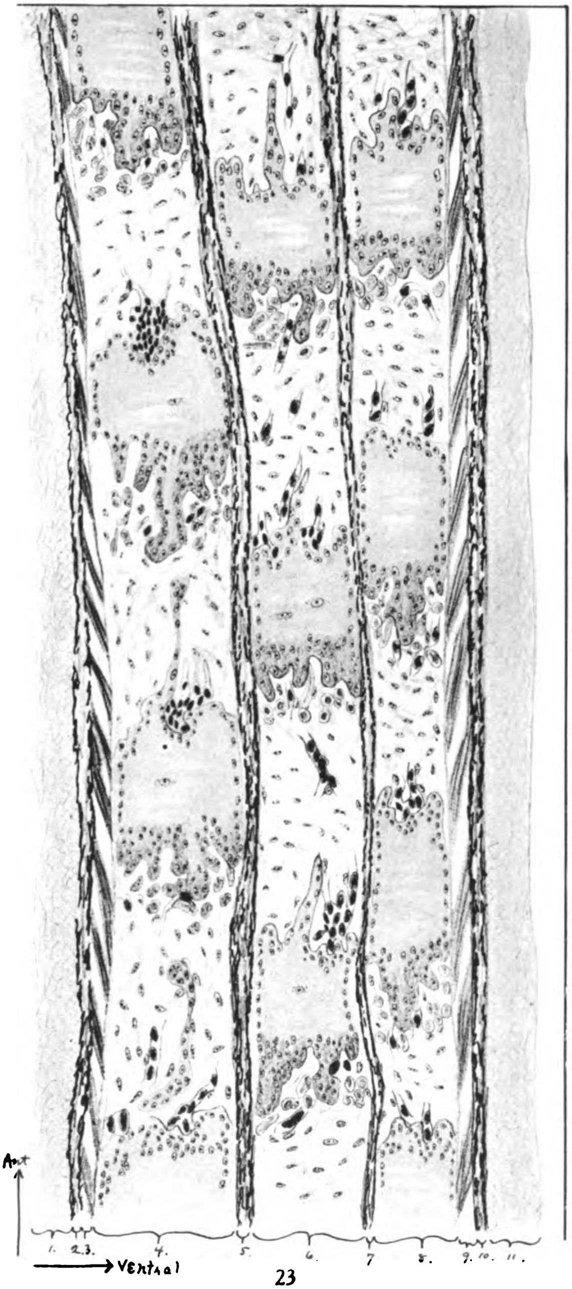

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XI.

THE DEVELOPMENT OF THE APYRENE SPERMATOZOA OF STROMBUS BITUBERCULATUS.

By EDWIN E. REINKE.
Proctor Fellow in Biology, Princeton University.

Seven plates.

CONTENTS.

Page

Introduction 197

Historical 198

Methods 205

Observations 206

  Adult spermatozoa 206

  Observations upon the living cells of the testis 210

  Growth of the apyrene spermatoblast 212

  Growth and differentiation of the apyrene spermatosome 221

Discussion 229

Summary 235

Bibliography 236

Explanation of figures 238

THE DEVELOPMENT OF THE APYRENE SPERMATOZOA OF STROMBUS BITUBERCULATUS.

By Edwin E. Reinke.

INTRODUCTION.

The fact that there is a dimorphism in the male sex cells of many Prosobrachs is well known at the present day. This is due to the great number of investigations that have been directed to the subject, but, in spite of them all, we are still as much in the dark as regards the function of the atypical spermatozoa as were the earlier investigators who did their work more than half a century ago. Furthermore, while the list of Prosobranchs in which this dimorphism obtains has been extended to a great many forms, in only a few of them do we have careful descriptions of the structure of the atypical spermatozoa, and in only one case (*Paludina*) is there a complete account of their development.

Obviously the solution of the problem is to be sought in a study of as many forms as possible, for I believe that each form has not only its own peculiarity in structure, be it great or small, but also its peculiarity in development. By peculiarity I mean not only the differences in form and size and in development between the typical and the atypical spermatozoa of any one genus, but also the differences between the atypical spermatozoa of different genera. In a comparative study of these peculiarities and differences may be found the explanation of the existence of the dimorphic spermatozoa. Comprehensive work such as this has already been begun; Kuschakewitsch ('11) has reported briefly upon the first two of a series of forms under investigation by him, and the writer has recently done the same for *Strombus*.

My attention was first called to the question of the dimorphic spermatozoa in Prosobranchs by Professor E. G. Conklin. At his suggestion *Urosalpinx cinerea* was made the main object of the investigation, supplemented by comparisons with the fresh-water snails *Goniobasis virginica* and *Paludina vivipara* (the Japanese variety). Material was collected and considerable work was done on these forms. But in May 1911, while at the temporary laboratory of the Carnegie Institution of Washington established at Port Royal, Jamaica, I was afforded the opportunity of studying and collecting material of various Prosobranchs, including *Strombus bituberculatus*. This form proved so interesting and so favorable for research that my immediate attention was centered upon it. My work was further advanced by another visit, in March 1912, to the temporary laboratory of the Carnegie Institution, this time at Montego Bay, Jamaica. Here I was able to carry on with partial success some experimental work and also to verify in the living cells most of the stages in the development of the apyrene spermatozoa that I had traced in preserved material.

My thanks are due to the Carnegie Institution for the privileges extended to me on both occasions, and in particular to Dr. A. G. Mayer, the Director of the Department of Marine Biology. Dr. H. A. Pilsbury of the Academy of Natural Sciences of Philadelphia very kindly identified the species for me. I am under great obligation to Dr. L. R. Cary, of the Department of Biology, Princeton University, for turning over to me his sectioned material of the testis and oviduct of *Goniobasis*. Finally, I wish to express a very sincere gratitude to Professor Conklin, not only for his many invaluable suggestions and criticisms, but also for the keen interest which he has at all times taken in the work.

HISTORICAL.

In 1837 v. Siebold discovered the fact that there are two kinds of spermatozoa in the testis of *Paludina vivipara*. The typical ones he called hair-shaped and the others worm-shaped spermatozoa. Since then the question of this dimorphism has been investigated by several writers nearly all of whom have given their attention mainly to *Paludina*.

Paasch ('43) and Kölliker ('41 and '47) denied the existence of the so-called worm-shaped spermatozoa of v. Siebold, the former interpreting them as being bundles of the hair-shaped spermatozoa and the latter claiming them to be different stages in the development of a single kind of spermatozoon. Leydig ('50), however, not only agreed with v. Siebold as to the existence of a dimorphism in the spermatozoa of *Paludina*, but also corroborated his description of the development of the worm-shaped kind. His views were very generally accepted until Duval ('80) investigated the question. He described an independent origin for the two kinds of spermatozoa, but in other respects his work was shown by v. Brunn ('84) to be quite inaccurate.

Although entirely confused in the successions of cell-generations, v. Brunn made a nearer approach to the facts in the case than anyone had done previously. He also described the dimorphic spermatozoa in five genera besides *Paludina* and later in the same year extended the entire list to include the following forms: *Paludina vivipara*, *Ampullaria* (sp.?), *Murex brandaris*, *M. erinaceus*, *M. trunculus*, *Cerithium vulgatum*, *Nassa mutabilis*, *Fusus syracusanus*, *Marsenia* (sp.?), *Aporrhais pes pelicani*, *Cassidaria echinophora*, *Dolium galea*, *Tritonium cutaceum*, *T. parthenopeum*, *Vermetus gigas*.

Brock ('87) added to this list by describing the atypical spermatozoa in three exotic Prosobranchs, *Pteroceras lambis*, *Strombus lentiginosus*, *Cypræa annulus*, *C. caput serpentis*, and *C. lurida*. As regards *Strombus*, his descriptions and observations are quite accurate as far as they go, but his figures are lacking in detail.

There then followed two papers, one by Köhler ('89) on *Murex*, and the other by Auerbach ('96) on *Paludina*. These authors established the fact that there is an almost complete loss of chromatin during the development of the worm-shaped spermatozoa, but they too were unable successfully to trace the succession of cell-generations and thus they made little or no material advance over the work of v. Brunn. To these observations Erlanger ('97) added a minute description of the adult worm-shaped spermatozoon in *Paludina*.

It is unnecessary here to go into a detailed discussion of the literature mentioned above, as it has all been discussed in the work of Meves ('03). This author has given a detailed and correct description of all the various stages in the development of both kinds of spermatozoa in *Paludina*. Furthermore, he has given careful consideration to the findings of the more important of the earlier writers and has shown wherein they were in error.

Meves describes the earliest differentiation between the two kinds of spermatozoa as occurring during the growth period of the spermatocytes, the spermatogonia of both being alike. He denies the statement of v. Brunn that the basal nuclei reproduce by direct division. This seems to the writer to be a debatable ground and forms the only weak point in the work of Meves. v. Brunn, of course, was quite wrong in supposing that the cells formed by this direct division of the basal nuclei give rise eventually to both kinds of spermatozoa; he failed to recognize the spermatogonia as distinct primitive elements of the testis. According to Meves these cells undergo unequal growth and thus give rise to both the hair-shaped and the worm-shaped spermatozoa. At the suggestion of Waldeyer he uses the terms eupyrene and oligopyrene to describe the two kinds of spermatozoa. The words have reference to their adult condition, *i. e.*, those provided with the ordinary amount of nuclear material and those with but a little; similarly, apyrene spermatozoa are those in which the entire nucleus disappears.

According to Meves, the oligopyrene spermatocytes of *Paludina* grow very much larger than the eupyrene and are clearly distinguishable, not only on account of their increased size, but also on account of their peculiar pattern and the presence of a large centriole and sphere. Preparatory to cell-division, this centriole divides and a little later both halves fragment into many smaller centrioles. The division which now occurs is abnormal. Two groups of very small centrioles form the poles of an ill-defined spindle. The daughter chromosomes pass to both poles, but before the division of the cell-body is completed the majority of them scatter again throughout the cytoplasm; four of the chromosomes, however, remain at the poles to form the new nuclei. These become vesiculated and may all unite with one another or some may unite and others remain single or all may remain single. Thus there may be four, three, two or only one nuclear vesicle in the secondary spermatocyte. The remaining ten chromosomes undergo a peculiar vesiculation in that the chromatin becomes lumped as a crescent on one side of the vesicle.

The second division follows immediately and is even more abnormal than the first. The centrioles scatter to the periphery and from each there pass out radiations. With the dissolution of the nuclear membrane or membranes, as the case may be, four new chromosomes make their appearance; these all show a longitudinal split. Very soon, radiations from a centriole are attached to each of the new chromosomes and they are drawn to various points near the periphery of the cell. By this time the ten vesiculated chromosomes remaining from the first division have been inclosed in a sort of capsule and are thus prevented from participating in the ensuing division. This is introduced by a slight elongation of the cell. Next, the centrioles move to the two ends of the cell and in so doing they form a spindle; one daughter chromosome then passes to each pole, while the other chromosomes remain scattered in the cytoplasm. With the division of the cell-body, the vesiculated chromosomes (inclosed in their capsule) pass into one or the other of the daughter cells, where they are set free by the dissolution of the capsule.

As a result of this division, Meves holds that the new nucleus is formed out of only a single chromosome, which very quickly becomes vesiculated and increases in size; all of the other chromosomes are gradually dissolved in the cytoplasm. Before the process of division is completed, cilia grow out from the centrioles, forming a brush at each pole of the dividing cell. After the cell has divided, the centrioles divide and their distal halves begin to move across the cell. A connection in the form of a fiber is always maintained between the proximal and the distal halves of the centrioles, the former remaining attached to the cell-wall. The nucleus in the meanwhile decreases in size and its chromatin forms a layer under the nuclear membrane. It moves closer to the plate of dividing centrioles. The continued movement of the distal centrioles across the cell results in pushing the nucleus against the cell-wall and in forming the bundle of axial fibers between the proximal and distal centrioles. Further growth of the axial fibers is accompanied by a gradual lengthening of the cell until the typical worm-shaped condition is reached. At the very end of the spermatozoon the nucleus forms a thimble-like cap over the end of the bundle of axial fibers.

In formulating an hypothesis to explain the problematical function of the oligopyrene spermatozoa, Meves discusses the theories of v. Brunn and Brock. v. Brunn believed them to be rudimentary eggs and therefore functionless, and this for two reasons: first, after careful investigation he failed to find them either fertilizing the ovum or even present in the oviduct; second, he believed their development bore resemblances to that of the ova. These views Brock attempted to refute on the grounds that any such rudimentary structure must have a proved homology with some still functioning part or organ and its departure in structure and development must be shown to be due to retrogressions; furthermore, if the dimorphism of spermatozoa is to point to an ancestral hermaphroditism, as v. Brunn claimed, the lower Prosobranchs should show this peculiarity, which they do not. He comes to the conclusion, then, that they are functionless.

Now Meves has shown that the oligopyrene spermatozoa of *Paludina* in their development show a marked parallelism—not to the ova, but to the true spermatozoa, and that they are produced in numbers equal to the latter. He suggests that there may be certain conditions under which they do fertilize the eggs; granting that they do and since the nucleus is accepted to be the bearer of heredity, then an embryo resulting from an egg fertilized by an oligopyrene or apyrene spermatozoon would show preponderating female characteristics. Such a case would prove the hypothesis that the nucleus is the bearer of heredity. The spermatozoon would then have, either primarily or solely, the mere function of stimulating the egg to division. According to Boveri, this stimulus is due to the introduction of a centrosome into the egg. But if it should be shown that actually the oligopyrene spermatozoa never fertilize the egg, then Meves would take the view, with Brock, that they are functionless.

Stephan ('03*a*) investigated the apyrene spermatozoa of *Cerithium vulgatum*, *Murex trunculus*, *M. brandaris*, *Triton nodifer*, and *Nassa mutabilis*. All of these forms he reported to have apyrene spermatozoa whose development is comparable to that of the oligopyrene of *Paludina*. All of the chromatin disappears in the atypical spermatozoa of these forms and in every case except *Cerithium* the bundle of axial fibers never comes into contact with an existing nucleus, as is the case in *Paludina*. In *Cerithium* the advancing bundle of axial fibers does touch a small nucleus which is the equivalent of a chromosome. Before the fibers have completed their growth, however, this small nucleus degenerates and disappears completely. Again, in *Cerithium*, as in *Paludina*, the cilia persist, while in the other forms they are retracted before the spermatozoon has fully matured. Upon these facts and also upon the positions of the anterior centrosomal structure in the adult atypical spermatozoa of the different forms under investigation, he concludes that gradations may be established between the different types of atypical spermatozoa, with *Cerithium* occupying an intermediary position between *Paludina* and the others.

In another paper Stephan ('03*b*) described briefly the development of the apyrene spermatozoa of *Murex brandaris*. The development here agrees in general with the description given by Meves for the oligopyrene spermatozoa of *Paludina*. It differs in the facts that there is an intense vacuolization of the cytoplasm and that there is no nuclear element in the adult spermatozoon.[1]

Basing his conclusions on certain observations of Popoff which were published later, R. Hertwig ('05) expressed his belief that the oligopyrene and apyrene spermatozoa serve to fertilize the egg, but such fertilization would not be a uniting of the male and female nuclear components, and would stand on the boundary between parthenogenesis and true fertilization.

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[1] A third paper by Stephan ('03*c*) on the development of the apyrene spermatozoa of *Cerithium vulgatum* and *Nassa mutabilis*, with two figures, was inaccessible to me.

This fertilization would result, according to Hertwig, in the production of males: "The sex-determining capacity which is indicated in the female by the formation of large eggs that are rich in yolk and small rudimentary ones, in this case would be transferred to the male sex."

Retzius ('06) described and figured the atypical spermatozoa of *Aporrhais pes pelicani*, *Turritella terebra*, *Murex trunculus*, *Fusus despectus*, and *Buccinum undatum*.

Popoff ('07) made a series of very careful and important observations upon *Paludina*. He examined the seminal receptacles of a great number of females at all times during the period of their sexual activity (May to November). He found that any one of the following conditions may obtain: (1) The receptacle may contain normal eupyrene and oligopyrene spermatozoa; (2) it may contain normal eupyrene and degenerating oligopyrene spermatozoa; (3) it may contain only eupyrene spermatozoa; (4) it may be completely empty. Of the 476 females examined during the whole period, in 176 cases the receptacle contained normal eupyrene and oligopyrene spermatozoa; the proportion of the cases in this condition to the total number examined at any one time showed a decided fall after the period of greatest sexual activity was reached (July). The condition which obtained in the next greatest number of cases (152) was the fourth, in which the receptacle was empty, and here the percentages of cases increased as the season advanced. In 89 cases the receptacle was found to contain only eupyrene spermatozoa, *i. e.*, the third condition; the percentage of these cases fell during August and then held fairly constant. Finally, only 39 individuals were found to be in the second condition (normal eupyrene and degenerating oligopyrene spermatozoa in the receptacle); here the percentage of cases gradually increased until August and then fell in September.

These facts indicated that the oligopyrene spermatozoa are shorter-lived than the eupyrene. In order to ascertain this definitely, Popoff segregated a number of females from the males immediately after copulation. From time to time the seminal receptacles of several of these females were examined and as a result he was able to establish the fact that after 10 to 12 days the oligopyrene spermatozoa begin to degenerate, while this does not occur among the eupyrene until after about 25 days.

In addition to this, Popoff found both kinds of spermatozoa in equal numbers in sections of the oviduct; only in the case of animals whose receptacles contained no oligopyrene spermatozoa did he fail to find these forms in the oviduct. This would indicate that the function of the oligopyrene spermatozoa is to participate in the fertilization of the egg. His results, however, in attempting to obtain eggs at the time of fertilization were practically negative. In three eggs, which constituted the only clear cases, Popoff found that the spermatozoa which had entered were eupyrene, so that nothing could be stated with certainty concerning the function of the oligopyrene spermatozoa. He thinks, however, if they may be said to have any function with regard to the egg (as is indicated by their presence in the oviduct), that this function can only be of a sex-determining character.

Lams ('09)[1] reported briefly that the development of the atypical spermatozoa in *Murex* confirms the description given by Meves for *Paludina*. In sectioned eggs he was able to find only eupyrene spermatozoa and therefore came to the conclusion that they alone serve to fertilize the egg.

The experiments of Kuschakewitsch ('10) have led to some very interesting results. By obtaining the ripe eggs of *Aporrhais pes pelicani* from the oviduct and mixing them with a mass of both kinds of spermatozoa which had been diluted with sea-water, he was able to observe the maturation and the first two cleavages of from 10 to 40 per cent of the eggs thus treated. In many instances he could see the formation of an entrance-cone and the entrance of the spermatozoon itself into the egg, but in no case was he able to observe the same processes for the atypical spermatozoa. But in sections of eggs which had been killed 20 minutes after mixing with spermatozoa, he found that the apyrene spermatozoa can and do enter the eggs, either alone or in addition to the eupyrene spermatozoa. Here the apyrene forms undergo a series of degenerative changes and are finally extruded. In later stages, during and after the first maturation spindle, their presence in the egg has never been detected.

Here it is seen, then, that the apyrene spermatozoa were extruded before any nuclear changes had taken place in the egg. Again, in some Prosobranchs the apyrene spermatozoa are very large and totally immotile, so that one can hardly imagine that they can in any way get into the egg. In view of these facts Kuschakewitsch has come to the conclusion that any sex-determining function in the apyrene spermatozoa is to be doubted.

Finally, Kuschakewitsch ('11) has briefly described the development of the eupyrene and the apyrene spermatozoa in *Conus mediterraneus* and of the apyrene spermatozoa in *Vermetus gigas*. In both cases the development of the apyrene spermatozoa shows many differences from that of the oligopyrene of *Paludina*. All three forms, however, have in common the greatly increased growth of the spermatocytes as the starting-point of the differentiation between the typical and the atypical spermatozoa.

Kuschakewitsch finds that in *Conus* no divisions occur in the development of the apyrene spermatozoa and that the spermatocyte is transformed into the spermatozoon by the disappearance of all the nuclear material and the subsequent growth of two fibers and two flagella from the centrioles. The nucleus may either gradually dissolve as a whole or it may fragment. The process of fragmentation may take place in either of two ways: the nucleus may become vesiculated and then break down into two and later into several compact and vacuolated spheres, or it may become compact and break down into a number of dense chromatic bodies which probably represent chromosomes. In either case the end result is the disappearance of all the chromatin. The two fibers eventually come to lie at the surface of the cell and the flagella with the pair of centrioles belonging to them at one of the ends of the spermatozoon. The conduct of the mitochondria is very striking; they appear first as granules, but eventually form fibers. During the course of development one or more vacuoles appear in the cell, which are quite characteristic of the adult spermatozoon.

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[1] I have been unable to obtain this publication and have therefore relied upon the review of it given by Kuschakewitsch.

In *Vermetus*, too, he finds that there is a direct breaking down of the nucleus of the spermatocyte. The chromatin forms into karyomerites and upon the dissolution of the nuclear membrane these lie free in the cytoplasm and are all gradually absorbed. In the spermatocyte there is to be seen a pair of centrioles lying in a thicker mass of cytoplasm; these form a group of secondary centrioles at one pole of the cell at the time of the dissolution of the nuclear membrane or immediately afterwards. The secondary centrioles give rise to a number of threads which eventually form a bundle of axial fibers whose middle portion passes through the center of the cell and whose ends extend beyond it in either direction. In the cytoplasm of the cell there arise a great number of thick-walled chambers whose cavities are filled with a clear substance. In each of these chambers a sphere of albumen is gradually formed. In the spheres themselves there appears either a large central vacuole or several smaller peripheral ones. Afterwards the contents of these vacuoles seem to become greatly condensed.

In his conclusions Kuschakewitsch points out the fact that the development of the atypical spermatozoa in *Conus* and *Vermetus* does not present the same parallel to the development of the typical spermatozoa as Meves established in the case of *Paludina*. He thinks, however, that we are justified in speaking of these structures as "spermatozoa" when the fact is borne in mind that their origin is the same as that of the oligopyrene spermatozoa of *Paludina* and *Murex* and that it is hardly conceivable that, in the well-defined group of Prosobranchs, structures with a similar origin would have a different morphological significance in different members of that group. Thus the mode of the development of the apyrene spermatozoa in *Conus* and *Vermetus* can in no wise be regarded as supporting the hypothesis of v. Brumm, that these structures are rudimentary ova. On the contrary, in *Conus* true eggs appear in the testis in addition to the apyrene spermatozoa.[1]

As regards *Strombus* the writer has shown (Reinke '12) that here, too, the development of the apyrene spermatozoa takes place without any maturation divisions, and furthermore that there are no processes, such as the formation of chromosomes and abortive spindles, which could be described as surviving from a condition in which those divisions did take place. In addition there is the fact (which will be shown in the succeeding pages) that the apyrene spermatozoa are not derived from the spermatogonia. But in spite of these facts, there are good reasons for still applying the term "apyrene (oligopyrene) spermatozoa" to these structures. In the first place, the name is well established and has been used commonly, and the words "apyrene" and "oligopyrene" are sufficiently descriptive to distinguish them from the true or "eupyrene" spermatozoa. Then again, they are spermatozoa in the sense that they are products of the testis and are found in the seminal fluid along with the true spermatozoa.

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[1] Since the above was written there has appeared another paper by Kuschakewitsch giving a detailed account of the development of the eupyrene and apyrene spermatozoa in *Conus* and *Vermetus*. In it he states that the apyrene spermatocyte of *Conus* sometimes undergoes a division after the nucleus has disappeared.

In retaining these names the writer does not mean to imply any homology between the apyrene and the eupyrene spermatozoa. Indeed, on account of the absolute lack of homology the terms "apyrene spermatocyte" and "apyrene spermatid" must be abandoned. A spermatocyte is a cell which is derived directly, by a mere process of growth, from a spermatogonium, and a spermatid is one of the four cells resulting from the two maturation divisions which such a spermatocyte has undergone and which is then transformed into a spermatozoon. The cells which give rise to the apyrene spermatozoa of *Strombus* go through two distinct periods of development and at no time can they be homologized with either the eupyrene spermatocytes or spermatids.

The first of these periods starts at the time when the cell is first recognizable in the testis.[1] This period continues throughout all the stages of the gradual but great growth of the cell and ends with the breaking down of the nucleus and the simultaneous scattering of the centrioles. The second period may be said to begin with the division of the centrioles; it is a period of marked cytoplasmic differentiations and it ends, of course, with the formation of the fully matured apyrene spermatozoon. For the cell during the first period it is proposed to use the name "apyrene spermatoblast" and during the second period to designate it as the "apyrene spermatosome." Both of these words, "spermatoblast" and "spermatosome," have been used hitherto in various connections; neither of them has the restricted meaning of "spermatogonium" or "spermatocyte" and, as used here, they imply no homologies with either of those structures.

METHODS.

Several fixatives were used for preserving the testis and the uterus of *Strombus*, namely, Bouin's, Flemming's, Helly's, and Zenker's fluids. Of these, Flemming's fluid (strong solution) gave by far the best results. Small pieces of the tissue were fixed for from 6 to 8 hours and were then washed for at least the same length of time, usually over night. As a result of such fixation the osmic and acetic acids did not penetrate very strongly into the inner portions of the tissue, and here the preservation of the mitochondria, the spheres, and the fibers was all that could be desired.

Sections were cut 5 micra thick and were stained with iron hæmatoxylin, followed by a weak solution of erythrosin in 70 per cent alcohol, which had been tinged to a purple color by the addition of a small quantity of a concentrated solution of gentian violet. The gentian violet was added to provide a nuclear stain for such structures as had not retained the iron hæmatoxylin; it gave a differential stain to the spheres and thus proved to be of considerable value. This stain has been used subsequently by other workers in this laboratory with fair success. Sections of the uterus and the seminal receptacle were stained in the same way.

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[1] It may be well to state at this point that the writer has succeeded in tracing the origin of these back to the so-called basal nuclei of Platner. He has found that the nurse-cells in the testis of *Littorina* have a similar origin, although the developmental changes are less complicated. It was deemed best, however, not to present the evidence of this origin at this time, but to wait until the history of the basal nuclei had been followed, in all its details, not only in these forms but also in other Prosobranchs, both those which have dimorphic spermatozoa and those which do not. The scope of the present paper, therefore, is confined to a description of the development of these cells from the time when their nuclei have been completely differentiated from the basal nuclei and have been established in the syncytium of the testis along with the true spermatogonia, each one surrounded by its own definite cytoplasmic body.

Smears from the testis, the vas deferens, and the seminal receptacle were prepared by making thin films of the contents of those organs diluted with sea-water. The films were allowed to dry over osmic vapor, were washed in fresh water, and then stained with Delafield's or iron hæmatoxylin.

Sea-water was found to be the best artificial medium for studying the living cells. The contents of the testis or sperm-duct were mixed with sea-water and then a drop of the mixture was placed on a slide and surrounded by a complete ring of glycerine-gelatine. In this way, a cover-glass placed upon the drop was not only supported, but it was also given enough rigidity to permit the use of an oil-immersion lense. If care were taken to see that the ring of glycerine-gelatine was unbroken, practically no evaporation of the mixture would take place. Under these conditions the various cells of the testis would remain alive for at least 2 hours. Sometimes methylene blue was used as an *intra vitam* stain, but this was found to be quite unnecessary and to cause an earlier breakdown of the cells than would otherwise occur.

OBSERVATIONS.

ADULT SPERMATOZOA.

The eupyrene spermatozoa of *Strombus bituberculatus* resemble in general those of other Prosobranchs. They are relatively short when compared with the spermatozoa of *Fasciolaria*, *Littorina*, or *Neritina*. The perforatorium is short and conical, the head thick and spindle-shaped (fig. 7). The head and the middle-piece, which is very slender and passes almost imperceptibly into the tail, constitute about half the total length of the spermatozoon.

In marked contrast to them stand the apyrene spermatozoa (fig. 6). The term "worm-shaped" can in no wise be applied to these, as they are totally different from the oligopyrene spermatozoa of *Paludina*. They were first described by Brock ('87) in the case of *S. lentiginosus* and again more recently and in some detail by the present writer ('12) in the case of *S. bituberculatus*. While Brock's brief description is correct as regards the general facts, his figure of the adult apyrene spermatozoon is not in accord with the facts as they have been observed by the present writer in *S. bituberculatus*, and there is no reason to suppose that any very great variation occurs between the apyrene spermatozoa of the two species. In their structure, the apyrene spermatozoa of *S. costatus* are identical with those of *S. bituberculatus*, the only difference being in their respective lengths. Apparently Brock did not fully understand the structure of the undulating membranes and he failed to observe the very evident centrosomal crescent at one end of the spermatozoon.

The adult apyrene spermatozoon of *Strombus* is composed of a central spindle-shaped body, which is long and narrow and slightly flattened dorsoventrally, on the sides of which are attached two broad undulating membranes (fig. 6). At the anterior end of the cell-body there is a crescent-shaped plate of centrosomal origin; starting from the horns of this crescent the undulating membranes round out quickly to their maximum width, while posteriorly they gradually become narrower and finally end in a short, sharply pointed tail-piece. In the membranes, here and there, can be seen indications of the fibers of which it is composed, but both these and the centrosomal plate are brought out more distinctly in fixed and stained specimens.

The interior of the cell is filled with a number of large secreted polygonal bodies composed of an albuminous substance or substances, possibly of a deutoplasmic nature like the yolk granules in the nurse-cells in the testis of *Littorina* (Reinke '12). When treated with iodine these bodies fail to give the reaction for starch or glycogen. When a mass of apyrene spermatozoa are separated from the eupyrene and are subjected to the xanthoproteic test, there is a very strong and clear reaction indicating the presence of albumen. In sections stained with iron hæmatoxylin the secreted bodies show the characteristic "Spiegelfärbung" of yolk granules. These bodies are more or less regular in shape and position but they decrease in size toward either end of the cell. In the living spermatozoon, at its posterior end, there can be seen a small aggregation of mitochondria. No traces of nuclear material can be found except in fixed specimens; here they appear as occasional darkly staining granules of degenerating chromatin lying between the albuminous bodies.

The orientation of the apyrene spermatozoon has been established upon morphological grounds. In *Paludina*, that end of the oligopyrene spermatozoon which contains the remains of the nucleus, *i. e.*, the head, has been designated as anterior and this, too, is the end toward which the axial fibers have grown. As there is no nuclear head in the apyrene spermatozoon of *Strombus*, the direction of growth of the axial fibers must determine the poles of the cell. The blunt, rounded end is the one toward which the fibers have grown and is therefore anterior, while the narrow pointed one is posterior. In movement it is the morphologically posterior end of the spermatozoon which is usually directed forward, although occasionally the reverse is seen to occur.

The movement of the spermatozoon is caused by contraction waves which pass alternately down the two membranes. At first these contraction waves are long and slow, propelling the spermatozoon with an even, steady motion. They usually pass down the membranes in a postero-anterior direction, but occasionally a reversal takes place; it is then that the spermatozoon is seen to move with its anterior end directed forward. The movement of the spermatozoon is comparatively slow and is not long continued, for it soon attaches itself, by means of its tail-piece, to the glass slide or other object on which it is being observed. As soon as this occurs, the contraction waves pass down both the membranes simultaneously and they then become much shorter and faster. With the spermatozoon attached in this way, the membranes may continue to be active for a time which varies with the medium used. In sea-water, contractions of the membranes were still seen after two hours, in isotonic NaCl after five hours, but in isotonic $MgCl_2$ they were very quickly inhibited. It very frequently happens, however, that the tail-piece breaks off and the spermatozoon swims away with a very much more rapid movement than it had at first.

When the tail-piece begins to break it can be seen to be composed of a number of fused flagella; sometimes, as the spermatozoon moves away a few of these flagella still adhere to it. This probably explains the statement of Brock to the effect that a tuft of flagella, which is invisibile at first, is to be seen after the spermatozoon has been swimming about for a while.

The first indication of breakdown in the spermatozoon occurs among the albuminous bodies, in that they gradually disappear. The process is undoubtedly one of liquefaction, for they leave in their stead a brownish semi-fluid substance in which, however, may still be seen the outlines of these bodies. When this has occurred one can observe for the first time the myoneme-like striations that are present on the cell-wall. Accompanying the disappearance of the albuminous bodies, there is a general flattening of the cell-body and the membranes. Undulations of the membranes continue a long time after this flattening has taken place and lead eventually to a partial separation of the membranes from the cell-wall. After this their disintegration is rapid.

The shape of the apyrene spermatozoa of *Strombus bituberculatus* is very constant and the variations in size that occur are very slight. From the centrosomal plate to the tip of the tail-piece the spermatozoa measure about 90 micra; the width of the membrane is usually about 5 micra and the greatest diameter of the cell-body averages about 8 micra. In *S. costatus* the apyrene spermatozoa are much longer, measuring 120 to 125 micra, but the other dimensions are not increased proportionately; according to Brock's statement they attain a length of 180 micra in *S. lentiginosus*. The apyrene spermatozoa in *S. bituberculatus* and in *S. costatus* are greatly outnumbered by the eupyrene. Brock has estimated the numerical relation between them to be about 1 to 500 for *S. lentiginosus*.

Because of the failure to obtain ripe eggs of *Strombus* at the time of the year when the material for this investigation was collected (March to the middle of May), it was impossible to determine experimentally whether or not the apyrene spermatozoa have any function with regard to the eggs. Ripe eggs of several sea-urchins were to be had in great abundance, however, and those of *Tripneustes* were mixed with some of the contents of the sperm-duct of *Strombus*. The presence of the eggs had no effect whatever upon either kind of spermatozoa except in a few instances where the egg-membrane had been ruptured by the pressure of the cover-glass. In such cases the eupyrene spermatozoa were strongly stimulated and swarmed around the eggs in great numbers, but the apyrene spermatozoa in the immediate vicinity of the eggs were not affected in the slightest.

At the same time of the year the seminal receptacle of the female was found to be quite swollen and full of a whitish mass. When some of the contents was diluted with sea-water and examined it was seen that in addition to a large number of oval granules only the eupyrene spermatozoa were present. These latter were normal and very quickly became active. Of a dozen females which were examined, not one showed a single structure in the seminal receptacle which could possibly be identified as the remains of an apyrene spermatozoon. The explanation of this phenomenon was found only when sections of the seminal receptacle had been made and studied.

The uterus and seminal receptacle constitute that portion of the vagina which lies just beyond the orifice of the oviduct, beneath the mantle. The vagina itself is simply a groove running beneath the mantle, at its base and on the right side, from the orifice of the oviduct proper to the foot; it corresponds to the genital groove of the male. Just below the orifice of the oviduct, the upper fold of the vagina is much broadened and thickened and is covered by the lower wall which is correspondingly broadened, forming a contractile sheath or mantle. This thickened portion of the vagina is the true seminal receptacle; it is a gland comprised of a large number of convoluted tubules lined with a secretory epithelium. The space between the seminal receptacle and its sheath may be spoken of as the uterus. Lying around the seminal receptacle in a semicircle is the main portion of the nidamental gland; one branch of the latter extends down along the vagina for a certain distance. The ducts of the nidamental gland open into the vagina, just below the seminal receptacle. Further on down, and at the point where the lower branch of the nidamental gland ends, is the opening into the vagina of a large diverticulum, the *bursa seminalis*. Beyond this point the vagina traverses the foot as an unmodified groove.

In the sections mentioned above, it was found that there was a dense detritus in the uterus composed of eupyrene and apyrene spermatozoa. Apyrene spermatozoa with relatively few eupyrene scattered amongst them formed the inner portion of this detritus. Segregating themselves from the apyrene spermatozoa, the eupyrene had moved for the most part to the periphery of the detritus. A clear homogeneous substance separated them from the apyrene spermatozoa. Other eupyrene spermatozoa had moved on into the tubules forming the seminal receptacle and had there arranged themselves with their heads against the outer surface of the epithelial cells. Here they were undoubtedly receiving nourishment, as was shown by the vacuoles which had taken the place of the distal granules of the secretory cells. In five series of sections no traces of apyrene spermatozoa were found in the tubules nor was either kind of spermatozoa found in the upper portion of the oviduct.

The changes that the apyrene spermatozoa had undergone were quite remarkable. In some cases the spaces between the albuminous bodies were packed with very small darkly staining granules; in others these granules had replaced the albuminous bodies in a portion of the spermatozoon; and in others the albuminous bodies had entirely disappeared and the whole body of the spermatozoon was filled with the small granules. In a few instances the body of the cell presented the appearance of an empty, shrunken shell lying between the two undulating membranes. Clearly the process going on in the apyrene spermatozoa was concerned with the breaking down and ultimate exhaustion of the substance stored in the cell.

It is probable, then, that in the examination of those dozen females only the tubules of the seminal receptacle had been tapped, and not the uterus, a contingency that is highly possible, depending upon the point of the incision and its depth. If that were so, then the granules observed were from the secretory granules of the tubules. But it is also possible that the uterus itself had been tapped and that the apyrene spermatozoa had undergone katabolic changes to such an extent that they were no longer recognizable.[1]

OBSERVATIONS UPON THE LIVING CELLS OF THE TESTIS.

By treating the contents of the testis in the manner already described, one can make a very comprehensive, if not detailed, study of the development of the apyrene spermatozoa of *Strombus*. This is particularly true of the later stages, where such structures occur as are represented by figures 1 to 5. These five figures, together with figure 6, show four very characteristic stages of the developing spermatosome from the time the axial fibers have grown across the cell up to and including the adult spermatozoon. They give a general idea of the appearance of the living cells and of the extent to which details of structure can be made out in them with the aid of an oil-immersion lens (Zeis 2 mm. apochromatic); furthermore, they bring out contours and the size relations existing between various stages to the greatest possible advantage.

In studying the living cells it was the hope of the writer not only to verify the stages which had been traced in fixed and sectioned material, but also, if at all possible, to watch the processes of growth and differentiation as they were taking place. All attempts, however, to observe the breaking down of the nucleus and the division of the centrioles proved to be unsuccessful. These processes are completed very rapidly and it is doubtful whether the centrioles with their radiations could be distinguished *intra vitam*. On the other hand, however, such processes as the vesiculation of the nuclear fragments, the formation of the albuminous bodies, and the growth of the axial fibers take place so gradually that they showed no appreciable progress in cells which were under constant observation for two or three hours. Nevertheless, many different stages in the development of the apyrene spermatozoa could be distinguished and observed most satisfactorily.

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[1] While a guest at the Laboratory of Marine Biology of the Carnegie Institution at Dry Tortugas during June 1913, the writer had the opportunity of examining some 25 females of *Strombus gigas*, all of which had copulated. While it is too late to present a detailed account of this examination, it may be stated that the results obtained give ample confirmation of the statements made above. The fates of the eupyrene and of the apyrene spermatozoa after copulation are very different. The eupyrene spermatozoa reach the seminal receptacle and are stored there in great numbers, remaining alive for an unknown period. Some of the apyrene spermatozoa move into the uterus along with the eupyrene, but there a sharp separation of the two kinds takes place, the eupyrene spermatozoa moving into the seminal receptacle while the apyrene clump together and undergo degenerative changes. Eventually the uterus becomes rid of them. But the greatest portion of the apyrene spermatozoa never even reach the uterus; instead, together with some eupyrene spermatozoa, they move into the *bursa seminalis* and there undergo degeneration until they are completely broken down. Gradually the whole mass is encapsulated in a secretion from the walls of the *bursa seminalis* and is finally extruded. In some instances it was found that the contents of the extruded capsule had become infected and that putrefaction had set in. There are some grounds for the writer's belief that some of the eupyrene spermatozoa which get into the *bursa seminalis* find their way out again and, passing up the vagina, reach the seminal receptacle. The most significant point is that oviposition had not yet begun.

The description given above of the relations of the various parts of the female genitalia is based upon dissections of *Strombus gigas*.

The large nucleated cell, corresponding to the oligopyrene spermatocyte I of *Paludina*, was easily recognized. Earlier stages in the growth of that cell could not be distinguished from the eupyrene spermatocytes. The next stage (fig. 1) which could be easily picked out was one in which the axial fibers had grown well across the cell and many of the nuclear fragments had become vesiculated. Figure 2 shows a stage of approximately the same age, but viewed at an angle of 90° from the other. In these stages the cell is not much larger than it was just previous to the breakdown of the nucleus. Later on, the continued growth of the axial fibers results in the elongation of the cell; this makes it appear larger, but no real increase in its volume has taken place. This is shown in figures 3 and 4, which represent stages following closely one upon the other and viewed in planes at right angles to each other. In figure 3 the bundle of axial fibers is seen just in the act of splitting apart, while in figure 4 the process is represented as being probably a little more advanced.

This splitting of the bundle of axial fibers is followed by the further separation of the halves until they reach the cell-wall on both sides. Very soon they begin to project edgewise beyond the cell, giving the first indication of the formation of the undulating membranes (fig. 5). Beyond this point there is not a very great increase, if any, in the actual volume of the cell; it merely becomes gradually longer and narrower until the adult condition is reached (fig. 6). It will be noticed that as the cell approaches maturity it becomes more and more filled with the albuminous bodies, while the nuclear fragments degenerate and disappear. When first formed, the albuminous bodies are spherical in size and are scattered irregularly throughout the cell, first filling up its anterior portion. Later on, as a result of their increase in number and the lateral compression of the cell due to its elongation, they become polygonal and arrange themselves in an orderly fashion. Until the undulating membranes have been formed the cell is immotile; the flagella at its base never beat, but instead undergo fusion to a certain extent. As will be shown later on, the myoneme-like striations are not present until after the formation of the membranes.

The study of the living cells of the testis is very valuable, because by this method all possibilities of abnormality due to fixation and subsequent treatment of the tissue may be eliminated. However, it leaves great gaps in the stages of development of the apyrene spermatozoa and affords very little or no chance of determining the finer details of the protoplasmic differentiations and the structural changes that occur in the cell. For this purpose the study of material that has been well fixed and stained is alone available.

GROWTH OF THE APYRENE SPERMATOBLAST.

In *Strombus* the large nucleated cell which becomes differentiated into the apyrene spermatosome can be traced back through a period of uninterrupted growth to a small cell, the apyrene spermatoblast, which is distinct from either the eupyrene spermatocytes or spermatogonia. This cell (fig. 8) lies close to the walls of the lobules of the testis and may be surrounded by older cells of the same nature or by the general syncytium of the testis or partially by both. Two or three of these cells, lying in the syncytium and isolated from others of their kind, are frequently in close proximity to a nest of eupyrene spermatogonia and would be taken at first glance to belong to that nest. Closer and more careful examination, however, reveals certain distinct, if minute, differences between them and the spermatogonia.

In the first place, there is a difference of position. Where they occur, the young apyrene spermatoblasts always lie between the wall of the lobule and the spermatogonia. If the group of apyrene spermatoblasts is a larger one, it is seen that the outer cells are larger and older than those lying next to the wall of the lobule. Such a group as that may be surrounded by several nests of spermatogonia or by spermatocytes. A comparison of all the nuclei of all these various cells shows that those of the outer spermatoblasts are as large or larger than the spermatogonial nuclei, while those of the inner spermatoblasts are smaller. But the cytoplasmic bodies of even the youngest spermatoblasts are larger than those of the spermatogonia and they are inclosed by definite membranes. The cytoplasm of the spermatogonia forms a thin sheath around their nuclei and the cell-walls are so delicate that very often it is impossible to distinguish them in the cytoplasm of the surrounding syncytium.

The nuclei of the apyrene spermatoblasts and of the eupyrene spermatogonia differ in pattern. In the latter, during the resting stages, the chromatin forms an irregular and dense network, the strands of which show many free ends and, when seen in cross-section, have very frequently the appearance of granules. Once the spermatogonia have begun to grow, the nuclear pattern changes gradually until the typical spireme of the spermatocyte is formed. The nuclear pattern of the spermatoblasts, on the other hand, remains constant until a late period in the growth of these cells, when they can be easily distinguished from the eupyrene cells by their increased size. These nuclei are relatively rich in nuclear sap and poor in chromatin, the latter being present in the form of irregular granules which are partially connected by a chromatic material (figs. 8–14). At least one karyosome is always present in the nucleus.

Finally there are two cytoplasmic structures which aid in distinguishing the young apyrene spermatoblasts. The first of these is a very evident, darkly staining granule, whose derivation is unknown. Its position varies; it may lie just beside the nucleus or nearer the periphery, but it is always found in the proximal half of the cell. It usually appears to be surrounded by a clear area (figs. 8 to 12). Occasionally a cell is found which has two or three of these granules in it. The other structure is the centrosome. This appears at first as a distinct centriole with a few faint rays and it lies between the nucleus and the base of the cell (fig. 8). In other spermatoblasts, which are still so young that they might be confused with the spermatogonia, it may be seen that the centriole has divided into two and that these are surrounded by a sphere (fig. 9). That centrosomes do exist in the spermatogonia is proved by their appearance in mitoses, but the writer has never been able to identify them positively in the resting cells. The distinct appearance of the centrosomes (centrioles) in the young spermatoblasts is not at all surprising when it is borne in mind that their later history is quite complicated and that they play a very important part in the development of the apyrene spermatozoa. The other bodies are never found in the cytoplasm of the spermatogonia. The eupyrene spermatogonia greatly outnumber the apyrene spermatoblasts and, with the basal nuclei, they occupy the space next to the wall of the testis except here and there where they have been displaced by groups of the spermatoblasts. These groups occur at irregular intervals and the cells composing them vary not only in number but also in age.

The development of the apyrene spermatozoa from these spermatoblasts takes place, as has already been indicated, without a single division of the cell. The first period of this development covers a gradual growth of the spermatoblast, resulting in a tremendous increase in the size of the cell. This statement applies not only to the nucleus and cytoplasm, but also to the centrosome; the latter comes to be the most striking and interesting element in the cell.

The structure of the primitive spermatoblasts has been described. They are irregular in outline and are not pear-shaped or stalked as are the oligopyrene spermatocytes of *Paludina* (fig. 8). Usually, as they increase in size and are crowded away from the wall of the lobule by the formation and growth of younger spermatoblasts, they are set free and tend to become round (fig. 12). Occasionally a cell which has almost attained its full growth is seen to have retained a connection with the syncytium by means of a stalk, even after it has been crowded away (fig. 13); such a cell presents a close resemblance to the oligopyrene spermatocytes of *Paludina*. In some few instances two or three fully grown spermatoblasts may be seen lying side by side close to the wall of the lobule and only separated from it by a thin layer of syncytium. These cells were the last ones to be formed at that point and have undergone their entire growth without suffering displacement. Both in the cases of these and of the stalked cells there is no relation between them and the syncytium other than one of close contact. The syncytium has become exhausted and has come to form a narrow fibrillar layer between them and the wall of the testis. This shrinkage of the syncytium beneath the spermatoblasts is noticeable in much earlier stages (figs. 9, 10, and 11).

The growth of the nucleus keeps pace with that of the cytoplasmic body. In the very young spermatoblasts the chromatin has the appearance of irregular granules lying in a matrix of diffuse achromatic material. There is always a karyosome present (fig. 8). Practically the same nuclear structures are shown in figures 9 and 10 except that in the former the karyosome was just above the plane of focus and in the latter the greater portion of the nucleus was in the next section. The karyosome is very persistent and constant in its appearance; in sections which have been destained until the other structures of the nucleus have lost their stain, the karyosome presents the appearance of a thick ring inclosing a lighter colored substance. As the nucleus grows larger, there is an increase in the amount of achromatin present and in the number of chromatin granules (compare figs. 10, 11, and 12).

The increase in the number of these granules is due, probably, to their division. The subsequent growth of the resulting halves keeps them all of a fairly constant size. When the nucleus has reached its maximum size the chromatin granules begin to grow at the expense of the achromatin (fig. 14). Their growth continues until they are as large or larger than the karyosome, so that the latter becomes indistinguishable. Eventually, practically all of the achromatin is absorbed and the chromatin granules are retracted to the periphery of the nucleus, just beneath the membrane. Here they undergo fusion to a certain extent and form large lumps of chromatin, or karyomerites, of various sizes and shapes (figs. 16, 17, and 18). The interior of the nucleus is filled with a colorless nuclear sap (fig. 17). It is now ready to break down, but before describing this process it will be well to consider the growth of the centrosome.

The first indication of a centrosomal structure in the spermatoblast appears at a very early stage, in the form of a distinct, darkly staining centriole, from which two or three rays (fig. 8) pass out. The position of this centriole, near the nucleus and in the proximal half of the cell, is invariable. The formation of a sphere around the centriole is not noticeable until the latter has divided (fig. 9). In such a stage as this the sphere is quite distinct and rays from both centrioles can be seen. It is impossible to say whether the next stage (fig. 10) is reached by a division of the two centrioles into four or by their fragmentation into a greater number. The centrioles are very much smaller than they were and can not be counted with any degree of certainty. The sphere has grown no larger and the rays from the centrioles do not extend beyond its periphery. The centrioles now lie in a homogeneous substance which fills the center of the sphere and is more deeply staining than the latter. This substance represents the first formation of the centrosome proper.

Further multiplication of the centrioles is accompanied by their separation from each other and by the spreading of the substance of the centrosome (figs. 11 and 12). It becomes very difficult to distinguish the individual centrioles from the substance in which they lie, but several of them can usually be seen lying at various points on the edge of the centrosome. The outer sphere, too, has increased in size and about this time begins to resolve itself into an outer dark court and an inner clear court (fig. 12). A little later on, the centrosome, which is now considerably larger, becomes spherical and acquires a definite membrane and the rays become stronger and stronger (fig. 13). It reaches its full growth at approximately the same time as does the nucleus (fig. 14). From it there passes out in all directions a great number of strong rays, which extend to all parts of the cell; at the base of each lies a small centriole. Lying around the centrosome on all sides, but more particularly on the sides away from the nucleus, is a mass of mitochondrial granules (figs. 14 and 15). They are irregular in shape and size and stain deeply with iron hæmatoxylin. Figure 14 does not show all the mitochondria that were seen in the cell.

The formation of the mitochondria is rather sudden. They appear in the sphere shortly after the formation of the inner court and are found at first on all sides of the nucleus. Figure 13 represents approximately the stage at which they first appear; it was drawn from a cell which showed the effects of osmic acid to some extent and therefore does not represent either the mitochondria or the rays as fully as they might be seen in other cells. As the mitochondria increase in number and size, they gradually obliterate the outer court of the sphere, while the inner court grows wider and wider. The last traces of the outer court are shown in figure 15. With the appearance of the mitochondria, one loses sight of that body which was mentioned as being found in the cytoplasm of the young spermatoblasts (figs. 8 to 12). This body gradually grows larger and very pronounced, but later on it is impossible to distinguish between it and any of the larger mitochondrial granules.

Judging from the great number of cells which are found with a fully formed centrosome, the last stage in the growth of the spermatoblast must extend over a very long period. Gradually the mitochondria withdraw from the space between the centrosome and the nucleus and that half of the centrosome which lies toward the nucleus becomes more lightly staining and seems to lose its definite outline. On the other hand, the opposite half of the centrosome takes a deeper stain and it can actually be seen that the centrioles are massing themselves upon that side (fig. 14). At this time, one or two comparatively large granules are to be found in the centrosome; they are very constant in their appearance but no suggestion can be made as to their significance. Finally, those rays which traverse the space between the centrosome and the nucleus attach themselves to the nuclear wall (fig. 14); then follow, in quick succession, the retraction of the chromatin to the wall of the nucleus, the dissolution of the latter on the side toward the centrosome, the scattering of the karyomerites, and the disappearance of the centrosome with its rays.

The actual beginning of dissolution of the nuclear wall is shown in figures 15, 16, 17, and 18; these go in pairs, figure 15 with figure 16 and figure 17 with figure 18, representing consecutive sections through two different cells. In figure 15 the mitochondria lie for the most part above the centrosome, while the latter in turn is at a slightly higher focus than the karyomerites. These last, represented in the figure as large black bodies, lie free in the cytoplasm, into which also some of the nuclear sap has escaped. Many of the mitochondria have grown into short stout rods (chondrioconts). It will be noticed that the rays from the centrosome are becoming less evident. In figure 16 is shown the remainder of the nucleus; some of the nuclear sap and a few of the karyomerites are moving out into the cytoplasm. The further and complete scattering of the karyomerites is usually accompanied by the total disappearance of the centrosomal rays. But in figures 17 and 18 a cell is shown in which absolutely no traces of the centrosome or the rays could be found and as yet none of the nuclear material had escaped into the cytoplasm.

These figures were drawn very carefully and show all the structures visible in the cell. The mitochondria were rendered somewhat indistinct by the action of the osmic and acetic acids, but the same can hardly be said of any surviving centrosomal structure; for a younger spermatoblast lying beside this cell showed a distinct centrosome. The nuclear membrane has become very much thinner and at one place, on the side towards the mitochondrial apparatus, it has been dissolved (fig. 18). It would therefore seem that the same process, undoubtedly a chemical reaction, which brings about the disappearance of the centrosome also leads to the dissolution of the nuclear membrane. Of the two phenomena, the latter is completed the more slowly. The karyomerites are carried to various parts of the cell with the diffusing nuclear sap.

The greater part of the chromatin remains in what was originally the distal half of the cell, although some of the fragments move into the region occupied by the mitochondria. This distribution of the chromatin is illustrated in figures 19 and 20, which represent two consecutive sections through the same cell. That part of the cell shown in figure 19 constitutes the proximal half of the cell, while the distal half is shown in figure 20. The larger lumps of chromatin now break down into several smaller parts (figs. 20 and 22), and as a result of this fragmentation there is a more equal distribution of the chromatin throughout the whole cell (figs. 24 and 25, consecutive sections through the same cell). The ultimate karyomerites show an extreme variation in size, some of them being extremely small (fig. 22).

Kuschakewitsch ('11) has described a somewhat similar formation of karyomerites in the apyrene cells of *Vermetus*. Here the karyomerites apparently are all fully formed before the dissolving of the nuclear membrane. They are of a fairly uniform size and shape and many of them show a longitudinal split, thus leading to the belief that they are true chromosomes. Kuschakewitsch states that "the chromatin of the nucleus gathers into larger elements, which almost entirely fill up the interior of the nucleus (Kernraum). In some cases there are formed, transitorily, true chromosomes which exhibit a clear longitudinal split. Then the nuclear membrane disappears and the chromatin elements (karyomerites) lie free in the plasma." The outstanding difference between the processes relative to the breaking down of the nucleus in the two forms is the fact that in *Strombus* the ultimate karyomerites are not formed within the nucleus while the latter is yet intact. Furthermore, when finally established, they have an extreme variation in size, little uniformity in shape, and never show any indications of a longitudinal split. In short, unlike those of *Vermetus*, the karyomerites of *Strombus* can not be said to be chromosomes, nor is it at all certain that they represent an attempt on the part of the chromatin to form chromosomes.

The further history of the karyomerites is one concerned with processes leading to their vesiculation and, later, to their gradual degeneration and ultimate disappearance. When first scattered into the cytoplasm the karyomerites lie in close contact with it on all sides. Very shortly afterwards, a clear space, one which gradually grows larger, separates them from the surrounding cytoplasm (figs. 20 to 25). The substance filling this space is essentially like nuclear sap and is probably formed by a differentiation of the cytoplasm immediately surrounding a karyomerite. A little later a definite membrane can be seen to have been formed around the clear area, separating it and the inclosed karyomerite from the cytoplasm (figs. 26 to 32). It is quite possible that this membrane is formed as a precipitation membrane between colloids of opposite electrical charges. If this explanation is the true one, then the differentiation of the cytoplasm must have been caused by a chemical reaction between itself and the karyomerite, the substance formed acquiring the acid properties of the chromatin. The reaction must continue until the interposition of the formed substance causes too great a separation of the reacting substances. Then, when the reaction has ceased, a precipitation membrane is formed between the acid and therefore positively charged substance resulting from the reaction and the basic and negatively charged cytoplasm.

After the formation of the membrane, the inclosed karyomerite breaks down into several smaller pieces, each of which takes up a position just beneath the membrane (figs. 26 to 32). In this way a number of chromatic vesicles arise which are very characteristic for the ensuing stages, and which, as will be shown later, persist for a long time before their actual degeneration sets in. Most of the smaller chromatic fragments, however, do not form vesicles but are absorbed without any further changes.

The disappearance of the centrosome takes place very suddenly and the nature of the process is not entirely understood. Absolutely no structures were found which could be interpreted with any degree of assurance as being phases of the centrosome subsequent to the stage shown in figure 15. One cell alone showed an indistinct structure which might be taken for the remains of a rapidly dissolving centrosome, but this can not be asserted to be the case (fig. 19). The body was round and apparently denser at its periphery than in the middle and from it there passed off a number of short and rather vague rays; it was partially hidden by what is taken to be a large mitochondrial body. The figure of it is not presented because much importance is attached to it, but merely because it happened to occur in a cell which was valuable for other features which it showed. All that is definitely known to occur is that while the substance of the centrosome is dissolved and disappears very rapidly, the centrioles themselves are unaffected by the action. Having been freed, they scatter in small groups.

The movements of the centrioles are hidden to a great extent, as it is very difficult to establish their identity among the many and very small mitochondrial and chromatic granules present. They eventually reach the cell-membrane, however, either singly or in small groups, and here some of them can be discerned quite readily (figs. 19, 24, and 25); others of perhaps questionable identity can sometimes be seen in the interior of the cell (fig. 19). A little later, secondary rays arise from those centrioles which have reached the membrane, causing them now to become very evident (figs. 21, 22, 26, and 27). Figures 21, 22, and 23 are consecutive sections through the same cell, and that portion of the cell represented by figure 21 showed the majority of the centrioles; those lay beneath the plane of the focus figured. A few others were found in the next section of the cell (fig. 22), but none were found in the upper section (fig. 23). It is thus seen that the centrioles come to lie on the periphery in one half of the cell and this half is the one which was originally proximal to the wall of the testis.[1]

The distribution of the centrioles was seen to better advantage in the cell represented by figures 26 and 27. Here they were found entirely confined to the left half of the cell. Those shown in the figures constitute probably less than one-quarter of the total number in the cell, but only those were figured concerning whose identity there could not be the slightest doubt. The same thing may be said of figures 28 and 29 which represent a slightly older cell. The centrioles are seen to have gathered into larger groups and have moved closer together. From each centriole, too, there has grown out a short flagellum. The rays from the centrioles were somewhat obscured on account of the "osmication" of the cell. These, however, are well shown in figures 30, 31, and 32, which represent consecutive sections through a cell of approximately the same age as the preceding one. This cell and others similar to it present the appearance of having polyasters on its one side, while on the other lies the majority of the now vesiculated karyomerites.

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[1] The fact that this half of the cell is the originally proximal one is easily established in the case of such spermatoblasts as have never been forced away from the syncytium. Four facts must be borne in mind: (1) in the young spermatoblasts the position of the centrioles is invariably in the proximal half of the cell; (2) matured spermatoblasts which lie close to the wall of the testis are independent and are not a part of the shrunken syncytium; (3) in such spermatoblasts the centrosome occupies a position distal to the wall of the testis; (4) they undergo their later development in the same position. It is impossible to believe that the centrosome and nucleus of these spermatoblasts have reversed their position during the growth of the cells. On the other hand, in view of the fact that these cells usually lie free except at the point of contact with the syncytium, it will be readily granted that the whole cell has probably revolved. Furthermore, a few instances of spermatoblasts in various stages of growth have been observed, the position of whose centrosomes would indicate a turning of the cell. Therefore, in these spermatoblasts, that portion which appears to be the distal half is really the originally proximal one. After the disappearance of the centrosome in such spermatoblasts as lie close to the wall of the testis, the centrioles come to lie beneath the membrane in the half of the cell directed toward the lumen, that is, in the originally proximal half.

This appearance is not maintained for any length of time, for the centrioles, with the lengthening of their rays, draw together and form a somewhat oval plate just beneath the membrane (fig. 33). The flagella now form a short, thick brush on the outside of the cell. The rays which have now grown very strong, traverse the cell for a short distance as a compact column and then, diverging, they extend to all parts of the cell. A little later the centrioles divide and then the distal halves begin to move across the cell, forming fibers between themselves and the proximal halves. The latter, which are perhaps smaller than the others, flatten themselves against the membrane, each one forming a node at the base of its flagellum.

The mitochondria can now be seen lying above the centrioles and encircling the rays which pass off from them. This irregular ring of granules is shown in cross-section in figures 33 and 35 and in surface view in figure 34. After the dissolution of the nuclear membrane and the dispersal of the karyomerites the mitochondria become obscured for a time, owing to the great difficulty of distinguishing between them and the smaller chromatic elements. They apparently remain in much the same position they occupied before the dissolution of the centrosome. With the vesiculation of the karyomerites they can once again be distinguished, but they now appear much more irregular in outline and size (figs. 28 and 29). Later they form the ring mentioned above.

The spermatoblast has now reached a condition in which it is ready to undergo the growth and differentiations which lead directly to the formation of the adult apyrene spermatozoon. With the completion of the primary growth of the cell, the nucleus has broken down to form a number of small chromatic vesicles. As will be shown later, these are concerned with the secondary growth of the cell, after which their degeneration sets in. By the accumulation of the centrioles at one point of the cell, and their subsequent division, the orientation of the matured apyrene spermatozoon is established; for, as has been pointed out, the antero-posterior axis of the spermatozoon is determined by the direction in which the distal halves of the centrioles traverse the cell. For these reasons the cell may now be spoken of as the apyrene spermatosome.

Before proceeding with the description of the growth and differentiation of the spermatosome, two facts must be considered concerning the spermatoblast: The first has to do with the polarity of the cell and the second with the lack of a strict time correlation between the breaking down of the nucleus and the disappearance of the centrosome, and between the phenomena immediately subsequent to both.

It has already been explained that the orientation of the apyrene spermatozoon is a morphological one, based upon the direction of growth of the bundle of axial fibers. It remains to be pointed out that this direction of growth is determined at the time of the appearance of the first centrosomal structure (the centriole) in the very young spermatoblast (fig. 8) and is parallel with an imaginary line passing through the centriole and the center of the nucleus. This line is the chief axis of the cell and it establishes not only the anterior and posterior poles of the adult spermatozoon, but also the plane of its bilateral symmetry. The centrosome is known to lie in the proximal half of the cell and upon its disappearance the centrioles come to be pretty evenly distributed on the periphery of the same half (figs. 26 and 27). If, in assembling, the centrioles move toward a central point, as there is every reason to believe, then the center of the plate formed by them will lie in the chief axis of the cell. After the division of the centrioles the distal halves move across the cell in a direction vertical to the plate and therefore parallel to the chief axis.

That the chief axis of the cell establishes a bilateral symmetry is shown by comparing figure 1 with figure 2 and figure 3 with figure 4. The first and third of these figures represent two different stages of the apyrene spermatosome, while figures 2 and 4 represent stages almost corresponding to them, but viewed at right angles to them. In the first case, a plane passing vertically through the longitudinal axis of the bundle of axial fibers divides either of the cells into like halves, while in the second case a similar plane divides either of the cells into unequal and dissimilar portions. The same bilaterality is established by the chief axis in the spermatoblast, indeed in very early stages (figs. 12, 13, and 14). Thus it appears that the polarity of the apyrene spermatozoon is determined at a very early stage in its development, at the time when the chief axis is established. The distal side of the spermatoblast marks the anterior end of the spermatozoon and the proximal side the posterior end. The latter may therefore be spoken of as the base of the spermatoblast or spermatosome.

While the dissolution of the nuclear membrane and the disappearance of the centrosome are initiated simultaneously, the phenomena immediately concerned with these two processes and also those subsequent to both do not keep step in different cells. It has already been pointed out that while in one cell (figs. 15 and 16) the centrosome was still perfectly visible after some of the karyomerites had escaped into the cytoplasm, in another (figs. 17 and 18) no traces of the centrosome could be found even before this had occurred. Similarly, in one cell (figs. 19 and 20) all of the centrioles have not yet reached the periphery and the larger karyomerites are fragmenting, while in another (figs. 21 to 23) the centrioles already show their secondary rays, although we are dealing with what is clearly an earlier stage as regards the chromatin. But in a third cell (figs. 24 and 25), and here the greatest variation occurs, we have a condition in which the centrioles have reached the periphery and their secondary rays have not as yet appeared, while the formation of the ultimate karyomerites has already taken place.

The occurrence of variations such as these seems to indicate that the processes concerned with the nucleus have great independence from those concerned with the centrosome and *vice versa*—greater than is known to exist in both normal and abnormal mitosis. In abortive mitoses, such as the so-called oligopyrene spermatocytic divisions of *Paludina*, there is always maintained a constant time correlation between the phenomena which occur in the nucleus and those which occur in the centrosome. An interpretation of the processes described above as being an abortive mitosis of the apyrene spermatoblast of *Strombus* seems to be unwarranted by the facts in the case.

GROWTH AND DIFFERENTIATION OF THE APYRENE SPERMATOSOME.

At first there is very little, if any, growth of the spermatosome; whatever growth there is, takes place slowly and for a long time but little difference in size exists between the spermatosome and the spermatoblast previous to the breaking down of the nucleus. The changes that occur are concerned chiefly with the chromatic vesicles and the centrioles.

By the time the centrioles have divided, the vesiculation of the karyomerites has been completed, or nearly so (figs. 33 and 34). The chromatic vesicles now acquire a very definite position in the cell. They lie in the outer portion of the cell, not far beneath the cell-membrane, and are confined almost entirely to the distal half of the cell. In a section passing through the chief axis of the cell, the chromatic vesicles are seen to lie in a semicircle, the open side of which points towards the base of the cell. This arrangement is shown particularly well in figure 39. At about this time, too, there is a very noticeable swelling of the chromatic vesicles due to the gradual increase in the amount of nuclear sap within them (fig. 41). In many instances an achromatic network is also visible in them (figs. 36, 39, 40, and 41). In a word, the chromatic vesicles now present the appearance of so many small but active secondary nuclei whose reconstruction is not yet completed; and such, indeed, they are considered to be by the writer.

It will be remembered that the secondary rays of the centrioles, at the time of the division of the latter, pass through the ring of mitochondria and then, diverging, they reach to the furthest parts of the cell. There is very little evidence in support of any belief that they attach themselves to the different chromatic vesicles; on the contrary, the ends of many of them can be clearly seen to pass on beyond the vesicles and lie free in the cytoplasm (figs. 33, 34, 35, 36, and 39). After the division of the centrioles, the connection of the rays with the distal halves of the former, or the distal centrioles as they will now be designated, becomes less distinct. In front of the advancing distal centrioles there is formed a dense and homogeneous substance which very frequently obscures those portions of the rays which pass through it (figs. 34 and 35). Into this substance, too, from the distal centrioles, there grow out slender fibrillar processes which become more pronounced as the centrioles advance (figs. 34, 35, 36, and 39). These for a time are probably continuous with the rays, but as the latter become less distinct whatever connection there may be between them is gradually lost (figs. 36 and 39).

As the distal centrioles move away from the base of the cell, there is formed between each pair of daughter centrioles an axial fiber (figs. 34, 35, etc.). These fibers are comparatively stout, more so than the flagella, and they stain very intensely with iron hæmatoxylin. In common with all the other centrosomal structures, they are brought out best in those portions of the tissue where the action of the acetic and osmic acids has not been too strong. In their journey across the cell, spinning out the axial fibers behind them, the distal centrioles come to pass through the ring of mitochondria (figs. 36 and 39). As they approach this ring they undergo a lateral compression in two opposite directions, so that they draw closer together and eventually fuse to form a solid, very flatly oval plate, whose greatest diameter is no less than that of the group of proximal centrioles at the base of the cell. The compression of the distal centrioles with the corresponding flattening of the bundle of axial fibers is shown in figures 36 and 39. It is shown to better advantage, perhaps, in figures 37 and 38, which represent two consecutive cross-sections through a bundle of axial fibers at about the stage shown in figure 39. The first of these cuts the axial fibers near the base of the cell and the second right through the ring of mitochondria; they bring out the difference in contour of the bundle of axial fibers at those two points and give some idea of the enormous number of fibers which constitute the bundle.

In front of the advancing centrosomal plate lies the area of dense material which was mentioned before and which is now becoming more and more evident (figs. 36 and 39). Around the anterior portion of the bundle of axial fibers lie other masses of denser cytoplasm, the inner margins of which are lined by the mitochondria (fig. 38). At this stage, or a little later, in many instances the bundle of axial fibers becomes somewhat twisted and the centrosomal plate is thrown a little out of its horizontal position. Further growth of the axial fibers is accompanied by a further flattening in its anterior portion and a more complete condensation of the centrosomal plate, so that the whole structure, when viewed from the side, has a sharply pointed appearance (fig. 40), while its upper and lower surfaces are as broad anteriorly as they are at the base of the cell (fig. 41). The dense substance in front of the centrosomal plate is pushed on ahead, while the other masses of dense cytoplasm, together with the mitochondria, spread out along the bundle of axial fibers behind the centrosomal plate, forming a sort of sheath around the former. The mitochondria are shown in longitudinal section in figure 40 and in surface view in figure 41.

As is indicated in figure 40, the spreading of the mitochondria and of the dense cytoplasm associated with them results in their separation into two parts, the posterior one of which comes to form a secondary ring around the base of the bundle of axial fibers. As before, this secondary ring is composed of the dense and homogeneous cytoplasm, the inner surface of which is bounded by the mitochondria. A cross-section through the posterior region of the spermatosome shows that the mitochondria of the secondary ring are closely applied to the bundle of axial fibers (fig. 42). The completed separation of the mitochondrial apparatus into an anterior and posterior portion is shown in figure 43. The peculiar structure of the secondary ring is maintained for a long time and the mitochondria connected with it persist in the adult spermatozoon. The mitochondria of the anterior portion, on the other hand, disappear very suddenly, at least no traces of them can be found after the stage represented by figure 43. It will be noticed that here already there has been a diminution of the denser cytoplasm around the anterior end of the bundle of axial fibers; by the time the centrosomal plate has reached the cell membrane practically all of it has disappeared (figs. 44 and 45). While it is possible that the anterior mitochondria may be dissolved *in situ* along with the other substance, two other explanations may be offered for their disappearance: (1) they may move in among the axial fibers and there give rise to occasional mitochondrial granules that are later found scattered throughout the length of the bundle or (2) they may be drawn back to the base of the cell and augment the secondary ring that has been formed there.

By comparing figure 42 with figure 37, it will be seen that after the formation of the secondary mitochondrial ring the posterior portion of the bundle of axial fibers has become more nearly circular, that its diameter is decreased, and that the fibers themselves have drawn closer together. The reduction of the diameter of the bundle is also shown in figures 41 and 42. The process seems to continue proportionately with the gradual growth of the fibers (figs. 44 and 46).

At first the growth of the axial fibers is very much slower than that of the flagella. It will be remembered that the flagella begin to grow out before the centrioles have divided. By the time the distal centrioles have traversed one-third the distance across the cell, the flagella have reached their maximum growth and are more than twice as long as the axial fibers (figs. 33, 34, 35, 36, and 39). From this point on they gradually become shorter and more ragged in appearance, eventually fusing at a very late stage to form the posterior tip of the spermatozoon (figs. 40, 41, 43–49, 63, and 64). This shortening of the flagella is probably due to their partial retraction into the cell, although their ragged appearance would suggest that they have been worn down by attrition with other free cells.

v. Brunn ('84) has described a similar action on the part of the flagella for the oligopyrene spermatozoa of *Paludina*. His observations were confirmed by Meves ('03), who explains the phenomenon on the ground that the flagella are the free ends of the axial fibers, each one passing through a ring formed by one of the proximal centrioles upon the cell-membrane. The shortening of the flagella is caused by the downward growth of the cell substance around and between them, so that, eventually, a portion of each flagellum comes to lie within the body of the cell. The facts that have been observed in the case of *Strombus* do not support this view. In the first place, there is not the slightest morphological evidence that the proximal centrioles form rings; they clearly form nodes just beneath the membrane and each one lies at the base of a flagellum. Then again, the flagella have begun to shorten before there has been any appreciable lengthening of the spermatosome in either direction; if any has occurred, it is certainly not sufficient to account for the very noticeable shortening of the flagella that has taken place.

It remains to be pointed out that there exists a striking similarity between the apyrene spermatosome (as regards its centrosomal structure at the time of the early growth of the axial fibers) and the well-known ciliated epithelial cells of the Mollusca. In figure 35, for example, the proximal centrioles can well be compared with the so-called basal granules of the ciliated epithelial cells and the distal centrioles with the inner granules. The slender fibers growing out from the distal centrioles are analogous to the fibers which extend across the epithelial cell. With the progressive growth of the axial fibers across the spermatosome the resemblance disappears to a great extent. While a strict homology between the two structures can not be maintained, we have here a strong, if indirect, support for the Lenhossék-Henneguy theory of the centrosomal origin of the basal granules found in a ciliated cell.

Several changes occur in the spermatosome about the time that the distal plate of centrioles reaches the cell-membrane. At this time or perhaps a little earlier, the chromatic vesicles begin gradually to lose their sap, so that they decrease in size and the chromatic granules come closer together (figs. 43–45). As the process continues the chromatin gathers on one side of the vesicle which is now becoming more and more reduced in size (figs. 46, 47, 48, and 49). Soon the membrane disappears, either by being dissolved or by the total loss of the sap, so that the whole structure shrinks into a solid mass. In either case, what were formerly active chromatic vesicles are now reduced to degenerating lumps of chromatin (fig. 63); eventually, all of these are dissolved in the cytoplasm and disappear, leaving no traces in the adult spermatozoon. The whole process of the degeneration of the vesicles can be followed through in the living cells (figs. 1–6).

The direct effect of the degeneration of the chromatic vesicles is found in the appearance at this time of the secreted albuminous bodies. Just as in some epithelial cells where the formation of the secreted granules is accompanied by the degeneration of the nucleus, so here the processes leading to the eventual disappearance of the chromatic vesicles go hand in hand with the secretion of the albuminous bodies. A similar correlation between the degeneration of the nucleus and the secretion of the albuminous bodies has been described for the so-called nurse-cells of *Littorina* (Reinke, '12). In *Strombus* the albuminous bodies appear first in the anterior portion of the spermatosome; a few can always be found around the advancing end of the bundle of axial fibers by the time it has grown across the cell. In later stages they are formed in increasing numbers and gradually come to fill up first the middle and then finally the posterior portion of the spermatosome (figs. 48, 49, 63, and 64; also figs. 1–6). By the time that all the chromatin has disappeared from the cell, practically all of the albuminous bodies have been formed (fig. 64).

The formation of the albuminous bodies takes place in very much the same way as Kuschakewitsch ('11) has described for the apyrene spermatozoon of *Vermetus* and which has been briefly reviewed on page 204. In *Strombus* there is not a general differentiation of the cytoplasm into a large number of thick-walled chambers. Instead, there is a continual formation of occasional vacuoles throughout the cell, those that are first formed filling up rapidly with the secreted albumen before the later ones have appeared. For this reason one never sees more than three or four vacuoles in any one given cell (figs. 43, 47, and 49). The extreme vacuolization shown in figure 46 is due to imperfect fixation and is not a natural condition. The process of the secretion of the albuminous bodies can be followed most readily in the posterior region of the spermatosome.

In a vacuole such as one of those shown in figure 49, a matrix is laid down composed of a substance which has a marked affinity for nuclear stains. The appearance of this matrix differs to some extent with the degree of "osmication" which the cell has undergone. In the inner portions of the tissue it is seen to be composed of a number of very irregular, darkly staining masses (fig. 52), while in the outer portion it appears to have the form of a network (fig. 63). It must be borne in mind that the magnification in figures 63–65 is less than in the preceding figures. Between these two extremes there stands a condition in which the network is made up of granules. Around this substance a clearer, homogeneous material is secreted, which gradually fills up the vacuole and effaces the matrix. In many instances narrow strands can be seen connecting this substance (an albumen?) with the surrounding cytoplasm (figs. 46, 49, and 54).

When the vacuole has been completely filled, a membrane is formed around the secretion, the body assuming a spherical shape. The bodies now give up their stain (iron hæmotoxylin) very readily; in the places where the spermatosome has been strongly "osmicated," they either appear unstained and yellow, or else they show the characteristic "Spiegelfärbung" of yolk granules. As has been stated, their change to the hexagonal shape seen in the adult condition is due to the pressure they exert upon one another after they have completely filled the spermatosome and the latter has undergone its final elongation and constriction.

By far the most striking changes that begin to take place at the time when the distal centrioles reach the cell membrane (figs. 44 and 45) are those which result from the continued growth of the axial fibers, viz., the formation of the undulating membrane and the resulting changes in the size and shape of the spermatosome. Having come to extend entirely across the cell, the bundle of axial fibers, by their continued growth, begins to protrude beyond the original boundary of the cell, causing the spermatosome to acquire a flask-shaped appearance (figs. 3 and 46). In the "neck" of the spermatosome, those yolk bodies which were the first to be formed come to lie between the broad surfaces of the bundle of axial fibers and the cell-membrane; the edges of the bundle, on the other hand, lie in close contact with the cell-membrane (figs. 3, 4, and 48).

At this stage, in the living spermatosome, there is always to be seen a very noticeable protuberance on one side of the brush formed by the flagella. This structure, although it is only a transitory one, is very constant in the living cells. The first indication of it is shown in figure 2, where it appears as a swelling at the base of the cell. Later it becomes very much longer and flattened in one axis (figs. 3 and 4) and appears finally to be withdrawn into the cell-body (fig. 5). The only trace of this protuberance that could be found in sectioned material is shown in figure 40. The point at which this protuberance occurs must mark the weakest part of the cell membrane; this is shown by the changes that occur when degeneration sets in. After a spermatosome in the stage represented by figures 3 or 4 has been in an artificial medium for two or three hours, the protuberance begins to become very much distended. This is followed by a shifting, to that quarter, of all the free contents of the cell, with the result that the bundle of axial fibers, instead of passing through the cytoplasmic body, lies tangentially to it and is surrounded by the cell membrane alone. This protuberance may be interpreted as a precocious lengthening of the cell in a posterior direction, the completion of which is prevented by the actual attachment of the fibers to the cell-wall. As will be shown presently, such a lengthening does take place at a very much later stage, but this time it is accomplished through the direct agency of the axial fibers themselves.

After a spermatosome has reached the stage represented by figure 46, a longitudinal split occurs in the bundle of axial fibers, dividing it into two approximately equal halves (fig. 47). This is caused by the continued growth of the axial fibers without a corresponding lengthening of the cell. Growth of the spermatosome does take place, to be sure, resulting in its becoming longer and narrower, but this growth is not commensurate with that of the axial fibers, nor is it accompanied by an increase in the volume of the cell. If these facts are borne in mind, then the following steps in the formation of the undulating membranes will be easily understood.

After the split occurs in the bundle of axial fibers, the two halves, or the secondary bundles, as they may now be called, move apart from each other, and gradually approach the cell-membrane from opposite sides. A lateral view of one of the secondary bundles, shortly after this movement has commenced, is shown in figure 48. By comparing figures 47 and 48, it will be seen that the albuminous bodies already formed are so placed that they do not obstruct the path of the fibers; those that eventually come to lie in the middle and posterior portion of the spermatosome are not secreted until after the fibers have reached the membrane on each side of the cell (figs. 49 and 63). As the secondary bundles separate, the individual fibers composing them fold back upon each other, so that several twists are formed in the bundles (figs. 49 and 63). Owing to the shape of the spermatosome, the secondary bundles reach the membrane in the anterior part of the cell long before they do so in the posterior region. Correspondingly, the differentiation of these structures to form the undulating membranes takes place progressively from the anterior to the posterior end of the spermatosome. Consequently, a series of cross-sections passing in the opposite direction through a spermatosome in the stage represented by figure 49 should show all the changes that take place both before and after the secondary bundles reach the surface.

Such a series is represented by figures 50 to 58. In figure 51 it is seen that the secondary bundles, in moving apart, have broken the ring of mitochondria which was mentioned before as having been formed around the base of the primary bundle. Afterwards, the mitochondria come to lie between the secondary bundles, while the dense cytoplasm which has been associated with them is gradually dissolved (figs. 49 and 63). It will be noticed that posteriorly the secondary bundles are wider than they are anteriorly. As they approach the surface the fibers draw closer and closer together; as a result, the bundles stain more and more deeply and it becomes increasingly difficult to distinguish the individual fibers. It can be seen, however, and very clearly too, that there is a modification taking place in that those fibers which lie nearest the cell-membrane tend to draw slightly away from the others and to fuse with one another (figs. 52 and 53). By the time the bundles have reached the surface, the fusion of these outer fibers has been completed and they are so closely applied to the cell-membrane that the latter becomes indistinguishable from them (fig. 54). The next figures show successive stages of the process by which the secondary bundles push out the cell-membrane and come to extend laterally beyond the body of the cell. Figure 58 represents a section passing just behind the centrosomal plate and shows that the fibers have arranged themselves into definite rows, a fact also indicated in figures 56 and 57. As will be shown presently, this arrangement of the fibers is necessary for the complete formation of the undulating membranes.

At a somewhat later stage, after the secondary bundles have reached the cell-membrane throughout their entire length, the myoneme-like striations are formed. This process is shown in figures 59 and 60. Some of the fibers which lie next to the cell-membrane now separate from their fellows and begin to move out across the surface of the cell just beneath the membrane. From this it would seem that there is not an actual fusion of the exterior fibers of the secondary bundles, but that they lie in very close contact with each other. It is probable, too, that the exterior fibers do not participate in the twisting of the secondary bundles spoken of above. Since the fibers are attached at both ends, the twisting of the secondary bundles must take place at their middle portions and doubtless proceeds in one direction and then in the other; it is simply an accompaniment of the separation of the secondary bundles and after these have reached the cell-membrane such twists as have taken place are apparently undone. At all events, as the secondary bundles begin to push out beyond the cell-body, a rearrangement of the inner fibers occurs in that they form a number of horizontal layers (figs. 56, 57, 58, and 60). Some of the inner fibers, apparently, crowd into the layer of exterior ones, taking the place of those that have moved away, while the others continue to form a decreasing number of horizontal layers. The result is that the bundles become wider and flatter. This is shown in cross-section in figures 61 and 62 and in surface view in figure 65. The first of these figures is an oblique section through the anterior region of a spermatosome at about the stage represented by figure 64. The section represents a cross-section just behind the centrosomal plate of another spermatosome of the same age. Figure 65 is a longitudinal section through a spermatosome which was almost identical with the one represented by figure 64. In this last figure the myoneme-like striations are also shown in surface view. As is shown here, the same force (that is, the continued growth of the axial fibers without a fully compensating increase in the length of the cell-body) which causes the secondary bundles to reach the surface of the cell and then to extend beyond it, eventually causes several folds to occur throughout their length. This is also indicated in figure 61.

After the secondary bundles have reached the cell-membrane, the space which has been vacated by them is filled with a substance whose consistency is greater than that of the cytoplasm surrounding the albuminous bodies (fig. 53). Indeed, there seems to be a general flowing of the denser, more granular ctyoplasm to the center of the cell, leaving the homogeneous enchylema to fill the spaces between the albuminous bodies (figs. 59 and 60). Eventually this denser part of the cytoplasm becomes differentiated in such a way that it forms a fibrillar core passing down through the center of the cell (fig. 64).

The changes in the shape of the spermatosome, as a result of the splitting of the bundle of axial fibers and the subsequent growth of these fibers themselves, are shown in figures 47, 49, 63, and 64. At first, accompanying the spreading of the secondary bundles to the surface of the cell, there is a slight elongation of the spermatosome in an anterior direction (fig. 49). This is continued until the secondary bundles begin to protrude beyond the cell-body, when it practically ceases (fig. 63). It will be noticed that the centrosomal plate has been pushed right up to the very anterior tip of the spermatosome. The further growth of the fibers results next in the lateral extension of the secondary bundles beyond the cell-body throughout their entire length and then in the elongation of the spermatosome in a posterior direction (fig. 64). This fact is evidenced not only by the change in the contour of the posterior end of the spermatosome, but also by the redistribution of the mitochondria. The base of the cell has become pointed and the flagella have fused to form the extreme end of the tail-piece. About this time, too, the folds that were mentioned before are formed in the secondary bundles.

A spermatosome which is in the stage represented by figures 64 and 65 is now ready to undergo the final change which transforms it into the fully formed apyrene spermatozoon (fig. 6). It has been completely filled with the albuminous bodies and all the chromatin has been dissolved in the cytoplasm; the secondary bundles have come to form folded ridges on both sides of the cell, already indicating the final shape of the undulating membranes. The length of the spermatosome, however, exclusive of the flagella is only about 70 micra. A general lengthening and constriction of the cell now take place which result in its becoming spindle-shaped, with an increase of from 15 to 20 micra in its length. At the same time the folds of the secondary bundles are somewhat straightened out and they themselves become much broader and flatter, assuming the shape and appearance of typical undulating membranes. The fibers are now probably arranged into two outer layers where they are in close juxtaposition to one another, if not actually fused, and a single inner layer where they are more widely separated from each other. The base of the cell is drawn out narrowly to a point, ending with the fused flagella. It is quite possible that the final change in the shape of the cell is to be attributed to the continued growth of the fibers.

DISCUSSION.

There are two outstanding differences between the development of the apyrene spermatozoa of *Strombus* and that of the oligopyrene spermatozoa of *Paludina*. The first of these is in regard to their origin. It has been shown by Meves ('03) that the oligopyrene spermatozoa of *Paludina* arise from the spermatogonia, certain ones of which undergo an unusual growth and become the oligopyrene spermatocytes. In *Strombus* the apyrene spermatozoa arise from certain cells which are different from the spermatogonia and which have been called the apyrene spermatoblasts. Secondly, in their later development, the apyrene spermatozoa of *Strombus* do not show nearly as close a parallelism to the development of the eupyrene as do the oligopyrene spermatozoa of *Paludina*. In both forms the development of the bundle of axial fibers in the atypical spermatozoon can be closely homologized with the growth of the axial fiber in the true spermatozoon, but the two divisions of the oligopyrene spermatocyte which take place in *Paludina* are entirely lacking in the apyrene spermatoblast of *Strombus*. Again, in the adult oligopyrene spermatozoon of *Paludina* the equivalent of one chromosome is retained and forms its nucleus, while the apyrene spermatozoon of *Strombus* contains no chromatin at all. Finally, it might be pointed out that the oligopyrene spermatozoon of *Paludina* shows a much closer approximation to the form of the eupyrene than does the apyrene spermatozoon of *Strombus*.

On the other hand, there exist certain fundamental similarities of development and structure between the atypical spermatozoa of *Strombus* and *Paludina* and indeed of other forms in which they have been described. The first of these is the relatively immense growth of the cell which ultimately gives rise to the atypical spermatozoon. Second, the history of the centrosome and the development of the axial fibers of the atypical spermatozoon present close parallels in *Strombus* and *Paludina*. The centrosome fragments and gives rise to a number of centrioles; the division of these with the subsequent separation of the daughter centrioles results in the formation of the bundle of axial fibers. In *Strombus* the differentiation is carried further, in that the bundle of axial fibers splits and the two halves move laterally to the surface of the cell and there form the two undulating membranes. Very similar phenomena have been described by Stephan ('03*b*) and by Lams ('09) for *Murex* and by Kuschakewitsch ('11 and '13) for *Vermetus*. In fact, in every form in which the development of the atypical spermatozoa has been described we find the differentiation of the centrosomal element into axial fibers.

Perhaps the most generally constant phenomenon in the development of the atypical spermatozoa of different forms is the fate of the chromatin. In *Paludina*, according to Meves ('03), one chromosome is retained and forms the nucleus of the adult oligopyrene spermatozoon, while all the rest of the chromatin degenerates. A similar condition is held by Lams ('09) to be the case in *Murex*, although Stephan ('03*b*) had previously reported that in this form all of the chromatin degenerates. In all other forms in which the matter has been investigated, the nucleus breaks down and an atypical spermatozoon is developed which contains no chromatin. Another characteristic held in common by the atypical spermatozoa of certain forms is the fact that the products of metabolism are stored in the cell. Thus, we find large secreted bodies in the atypical spermatozoa of *Strombus*, *Pteroceras* (Brock, '87), and *Vermetus* (Kuschakewitsch, '11 and '13). It is probable, too, that the mitochondrial apparatus, so beautifully described and figured by Retzius ('05), in the oligopyrene spermatozoa of *Paludina* is of the same nature, as well as the refractive bodies described in the atypical spermatozoa of *Cypræa* by Brock ('87), of *Murex* by Koehler ('88), and other writers and the vacuoles in the apyrene spermatozoa of *Conus* described by Schiemenz ('96) and Kuschakewitsch ('11 and '13).

Both Stephan ('03*a*) and Kuschakewitsch ('11) have suggested the possibility of arranging the atypical spermatozoa of different forms into a gradated series based upon differences in their development and structure and representing further and further retrogressions from the typical spermatozoa. At the head of such a list would be placed *Paludina* and *Murex* in the order named. Next would come *Conus*, for here Kuschakewitsch ('13) has recently found that one division normally occurs in the development of the apyrene spermatozoon. After these there might follow in succession *Cerithium*, *Vermetus*, and *Strombus*. The list can not safely be extended further than this, since we are not sufficiently acquainted with the phenomena which take place in other forms; and even as it stands the list is full of contradictions. Thus, for instance, *Cerithium* should perhaps precede *Conus*, since in the former one chromosome is retained in the apyrene spermatozoon for a longer time than the others (Stephan, '03*a*), while in *Conus* all the chromatin of the apyrene spermatocyte degenerates simultaneously. Again, when considered from the standpoint of the differentiations of the centrosome and the development of a motor apparatus, *Conus* should be placed at the end of the list, for here the apyrene spermatozoa are totally immotile. *Vermetus* has been placed before *Strombus* because of the formation of chromosomes and of an abortive spindle in the development of the apyrene spermatozoa of the former; but the order could well be reversed when it is borne in mind that, in the development of the bundle of axial fibers of the atypical spermatozoon, *Strombus* resembles *Paludina* much more than does *Vermetus*. The truth of the matter is that we do not have sufficient data upon which to formulate such a series. In the writer's opinion, the evidence is not clear enough for us to draw correct inferences as to whether or not we are dealing with a series of retrogressions from the atypical spermatozoa.

While it has been shown that the atypical spermatozoa, in all those forms in which they have been studied, show certain fundamental similarities, each one also has certain differences peculiar to itself. One of the greatest of these differences, perhaps, is that in regard to the origin of the apyrene spermatozoa of *Strombus*. Nevertheless, in spite of such differences, it is the writer's opinion that they are all essentially the same and are derived from similar elements of the testis—that is, they are modifications of the accessory cells of the testis, just as are the nurse-cells in the testis of *Littorina*. It is suggested that the explanation of the differences between them is to be found in the manner in which the differentiation of the primitive cells of the sexual gland takes place into the various elements of the testis. So, in the Pulmonates there is a sharp differentiation of the primitive sex-cells into the true sex-cells and into the accessory cells, the basal nuclei with their surrounding syncytium. Here the latter never become differentiated into independent cells, although they undergo changes in their size and shape (Platner, '85). Among the Prosobranchs, in *Littorina*, the writer has never observed the same tremendous swelling of the basal nuclei as may be seen in the ovotestis of *Planorbis*, but, instead, certain of them give rise directly to the nurse-cells to which the spermatozoa are attached. In *Strombus* those elements which give rise to the atypical spermatozoa are differentiated from the true sex-cells along with the basal nuclei. The latter never give rise to nurse-cells of any kind. In forms such as *Paludina* and *Murex*, the elements which give rise to the atypical spermatozoa are not differentiated from the true sexual elements along with the basal nuclei, but only after the spermatogonia have been formed. In *Paludina* the formation of nurse-cells has never been observed by the writer nor have they been described by Meves, but the basal nuclei undergo changes which are very similar to those which occur in the ovotestis of

Planorbis. In *Vermetus* and in *Conus*, too, according to Kuschakewitsch ('13), the atypical spermatozoa are not differentiated until after the formation of the spermatogonia. But in both these forms nurse-cells are also developed and apparently from the basal nuclei; whether or not their development is the same as that of the nurse-cells in *Littorina* is not known by the writer. In all these forms there is a period in the development of the testis when the true sex-cells are undifferentiated from the accessory elements. It may be that certain differences in the plasma of the primitive cells lead to differences in the manner of differentiation of the accessory elements and these in turn lead to the production of adult structures which differ from each other to a greater or less degree, but all of which show certain fundamental resemblances.

Besides the characteristics held in common between the atypical spermatozoa of *Strombus* and those of other forms, the former show certain resemblances to the nurse-cells in the testis of *Vermetus* and of *Littorina*. While there are no large cells in the testis of *Strombus* which could possibly be taken for anything except the apyrene spermatoblasts, there exist a striking resemblance between certain of the developmental stages of these and some of the figures given by Kuschakewitsch ('13) for the nurse-cells in the testis of *Vermetus* (figs. 104 and 105, Taf. XXIV, and figs. 169 and 170, Taf. XXV, Arch. f. Zellforsch., Bd. X). The writer has shown that nurse-cells are developed in the testis of various species of *Littorina* which, when they have reached their maximum size, resemble the oligopyrene spermatocytes of *Paludina*. Later on, the cells become filled with large secreted bodies, while their nuclei gradually degenerate. In the final stages the latter may still be seen in a very shrunken, degenerated condition lying just beneath the cell-membrane. It is in connection with the formation of these secreted bodies and the corresponding changes in the nucleus that the apyrene spermatozoa of *Strombus* resemble the nurse-cells of *Littorina*. It will be remembered that in the apyrene spermatoblasts, after the nucleus breaks down, chromatic vesicles are formed which gradually increase in size until the formation of the albuminous bodies is begun. From this point on, with the continued formation of the albuminous bodies, they gradually degenerate and are finally all absorbed in the cytoplasm.

A comparison of the processes which occur on these cells and those which take place in normal secretory cells lead to some interesting considerations. Mathews ('99) has shown that secretory activity or, to use his term, hylogenesis, is accompanied in various epithelial cells by certain changes in the nucleus, in that the latter becomes irregular in outline and is displaced towards the base of the cell. In these cells the process is cyclic; after the cell has been exhausted the nucleus returns to its normal condition and the activity begins over again. The changes which the nurse-cells of *Littorina* undergo at the commencement of their secretory activity are very similar to those which take place in secretory epithelial cells. In the former case, however, the process is not repeated and in every instance it is accompanied by the gradual degeneration of the nucleus. It seems to be not unlikely that these two facts stand in a causal relation to each other.

The same condition, that is, the unrepeated secretory activity of the cell accompanied by the degeneration of the nucleus, holds true in the case of the apyrene spermatozoa of *Strombus* as well as in the nurse-cells of *Littorina*. But here the question might well be asked, why does not the nucleus degenerate directly, instead of undergoing the complicated history that has been described? The primary function of the nucleus is concerned first with the growth of the spermatoblast and then with the secretion of the albuminous bodies, and possibly its presence is essential for the development of the great centrosome with its many centrioles. The differentiations which occur in connection with the centrioles lead to the formation of the undulating membranes and they are initiated by the disappearance of the centrosome. It is believed that the impulse, whatever it may be, which is responsible for the disappearance of the centrosome causes also the dissolution of the nuclear membrane. That impulse may be the same as that which causes a cell to divide mitotically, but, as has been shown, there is no real evidence to support such an assumption. Stated a little differently, when considered in regard to the subsequent development of the apyrene spermatosome, it is the change that occurs to the centrosome that is of prime importance at this time and that which occurs to the nucleus is incidental to it.

Were this not so, we should expect either that the chromatic fragments would degenerate without first becoming vesiculated and the secretion of the albuminous bodies be commenced at the same time, as is the case in the apyrene spermatozoa of *Vermetus*,[1] or else that the nucleus would remain intact until the secretory activity of the cell is begun and then degenerate as a whole, which is the case in the nurse-cells of *Littorina*. Instead, the karyomerites become vesiculated and form a great number of secondary nuclei which increase in size until the first albuminous bodies are formed, when they begin to degenerate. Moreover, if the secretory activity of the cell is enhanced by an increase in the surface contact between the nucleus and the cytoplasm, then the formation of the secondary nuclei is of great advantage.

The conditions observed in the seminal receptacle and uterus of *Strombus bituberculatus* and *S. gigas* after copulation and at a time before oviposition has commenced, and indeed before the ova are fully matured, throw a certain amount of light upon the nature of their function. The fact that eupyrene spermatozoa are found in those parts at such a time indicates that they, or at least some of them, must remain there for a protracted period—that is, until and during the time of oviposition. The greater part of that time is no doubt spent in the seminal receptacle, where they can and do receive an independent supply of nourishment. But, judging from the fact that in nearly every case examined they have also been found in the uterus along with the apyrene spermatozoa, it must take a long time for them to move into the seminal receptacle. In the uterus a separation of the two kinds of spermatozoa occurs, the eupyrene being retained in good condition while the apyrene may be seen in all conditions of katabolic changes leading to the total exhaustion of the secreted bodies. It may be that the apyrene spermatozoa serve to nourish the eupyrene until the latter are stored in the seminal receptacle, where they may obtain an adequate and independent food supply. Whether this is so or not, it is believed that the apyrene spermatozoa of *Strombus* subserve their purpose in the uterus and possibly also in the vagina and bursa seminalis immediately after copulation and that their function is connected with their breaking down.

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[1] In this connection Kuschakewitsch ('13) has made the suggestion that in *Vermetus* the retention of the chromatic elements as such almost until the end of spermatogenesis is to be explained on the grounds that the chromatin participates in the formation of the albuminous bodies in the protoplasm of the apyrene spermatozoon.

There are two other ways of explaining the behavior of the apyrene spermatozoa after copulation has taken place. The first of these was suggested to the writer by Professor Conklin and is to the effect that the apyrene spermatozoa in breaking down may liberate a substance which stimulates the eupyrene spermatozoa or the eggs or both during fertilization. The possibility of their being an aid in the final disposition of the eupyrene spermatozoa was indicated by a series of experiments recently performed by the writer.[1] It may be that the substance liberated by the apyrene spermatozoa as a result of the changes which they undergo causes the eupyrene spermatozoa to withdraw from them and eventually to enter the seminal receptacle.

The fact that the apyrene spermatozoa of *Strombus* break down after copulation and are discarded, while the eupyrene are retained and kept in a healthy condition, together with the results obtained by Kuschakewitsch ('10) in his study of the fertilization of the eggs in *Aporrhais pes pelicani*—these facts, in the writer's opinion, preclude the view that the atypical spermatozoa may participate as such in the fertilization of the egg.

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[1] While at the Tortugas Laboratory during June 1913, cultures of the contents of the sperm-duct of *Strombus gigas* were kept alive and observed under varying conditions. The spermatozoa lived longest in cultures made from sterilized sea-water, in some cases as long as 72 hours. The length of life of the spermatozoa apparently depended upon the extent to which the cultures had been kept free from bacterial infection. In such cultures, there was always a separation of the two kinds of spermatozoa, the eupyrene remaining active for a long time after the apyrene had ceased their movements. In very many instances a clear drop of some substance could be seen attached to the side of an apyrene spermatozoon whose contents had partially or completely broken down. Very striking results were obtained in a few cultures in which a small piece of decaying tissue was included in order to test the reaction of the two kinds of spermatozoa to CO_2. In every case the eupyrene spermatozoa moved further and further away from the source of the CO_2 until they reached the edges of the cover glass. The apyrene spermatozoa, on the other hand, were not greatly stimulated except in one instance, where they gave a distinct, positive reaction, gathering around the pieces of decaying tissue. One effect of CO_2 upon the apyrene spermatozoa was to cause a comparatively rapid and complete breakdown of their contents. Unfortunately, for lack of time, these experiments could not be repeated under conditions of greater accuracy.

SUMMARY.

1. The apyrene spermatozoa of *Strombus*, in the adult condition, are devoid of any nuclear material. The body of the spermatozoon is spindle-shaped and is filled with large hexagonal albuminous bodies. Locomotion is brought about by means of the undulations of two broad membranes attached to each side of the body.

2. Together with the eupyrene spermatozoa, they are found in the uterus of the female, where they undergo certain katabolic changes resulting in the total exhaustion of the albuminous bodies. They are never found in the seminal receptacle, even when the latter is crowded with eupyrene spermatozoa.

3. The differentiation between the eupyrene and the apyrene spermatozoa can be traced back further than the growth period. In fact, apyrene spermatozoa develop from cells which can be distinguished from the spermatogonia of the first generation and which have been named the apyrene spermatoblasts.

4. The apyrene spermatoblasts are believed to arise from the basal nuclei. Confirmation of this phenomenon in other related forms is required before it can be considered an established fact.

5. The very early appearance of a centriole in the apyrene spermatoblast establishes the polarity of the apyrene spermatozoon.

6. The growth of the apyrene spermatoblast is marked by a great increase in the volume of the cytoplasm and nucleus and by the development, from the original centriole, of a very large centrosome containing many secondary centrioles at its periphery. No division of any kind takes place during the development of the spermatoblast.

7. After the apyrene spermatoblast has acquired its full growth, the dissolution of the nuclear membrane and the disappearance of the centrosome take place simultaneously. The chromatic fragments (karyomerites) are scattered throughout the distal half of the cell and the centrioles become grouped beneath the membrane at the base of the cell. From each of the latter a flagellum grows out. The cell may now be spoken of as the apyrene spermatosome, for the ensuing changes give rise directly to the adult apyrene spermatozoon without any appreciable increase in its volume.

8. The centrioles divide and the distal halves move across the cell in the direction of the chief axis, forming a bundle of axial fibers between themselves and the proximal halves. At the same time the karyomerites become vesiculated and remain active for a time, gradually becoming larger.

9. The mitochondria which have been formed around the centrosome during the growth period now constitute the inner margin of a ring of dense cytoplasm which lies around the bundle of axial fibers at its base.

10. When the bundle of axial fibers has come to extend entirely across the cell, the secretion of the albuminous bodies is begun in the anterior portion of the spermatosome. This process continues hand in hand with the degeneration of the chromatic vesicles, until, successively, the middle and

posterior portions of the cell have been completely filled with them and all of the chromatic vesicles have disappeared.

11. In the meantime, the bundle of axial fibers continue to grow until the cell has been elongated in an anterior direction, along its chief axis, by about one-third its original diameter. Afterwards, the continued growth of the axial fibers is not accompanied by a fully compensating increase in the length of the cell; the changes which ensue are all the direct result of this fact.

12. A longitudinal split occurs in the bundle of axial fibers, dividing it into two equal halves, each of which moves in an opposite direction towards the surface of the cell. In doing so they rupture the ring of dense cytoplasm which lie around the base of the bundle. This substance gradually disappears, but the mitochondria associated with it persist in the adult apyrene spermatozoon.

13. Having reached the surface of the cell, the secondary bundles of axial fibers now come to extend laterally beyond it. The further growth of the fibers causes a number of folds to occur throughout the length of the bundles. Finally a general elongation and constriction of the cell occur, with the result that the body of the spermatosome acquires the spindle-like shape of that of the adult apyrene spermatosome and the secondary bundles on both sides of it become straighter and more flattened, forming the undulating membranes.

14. In the writer's opinion the apyrene spermatozoa of *Strombus* subserve their purpose after copulation has taken place and their function is connected with the katabolic changes which they undergo. Their behavior may be explained on the grounds of the following three suggested possibilities: (*a*) They may serve as nurse-cells to the eupyrene spermatozoa after copulation and before the latter reach the seminal receptacle; (*b*) they may, by the liberation of some substance, stimulate the eupyrene spermatozoa or the eggs or both during fertilization; or, by the liberation of some substance to which the eupyrene spermatozoa are negatively chemotactic, they may act as an aid in the final disposition of the latter.

15. The conditions met with in the uterus and seminal receptacle of the female after copulation has taken place and before oviposition has commenced show that the apyrene spermatozoa do not participate as such in the fertilization of the egg.

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EXPLANATION OF FIGURES.

All figures were drawn at table height with the aid of a camera lucida. With the exception of those on plate 1 and figs. 63–65, all figures were drawn with the use of the Zeiss 1.5 mm. apochromatic objective and the 12 compensating ocular; the figures on plate 1 were drawn with the use of the Zeiss 2 mm. apochromatic objective (N.A. 1.30) and the 6 compensating ocular and were then enlarged to 1825 diameters, while figs. 63–65 were drawn with the use of the Zeiss 1.5 mm. apochromatic objective and the 8 compensating ocular. In studying the cells the Zeiss 1.5 mm. apochromatic objective as well as the 2 mm. (N.A. 1.40) were used in combination with the 8 compensating ocular. Figs. 8–65 were drawn from sections, 5 micra thick, of material fixed in Flemming's fluid (stronger solution) and stained with iron hæmatoxylin followed by gentian violet and erythrosin.

PLATE 1.

(Initial magnification 1825 diameters, not reduced.)

1 to 5. Three progressive stages in the development of the apyrene spermatosome, drawn from living material. Figs. 1 and 2 show corresponding stages viewed in planes at right angles to each other; figs. 3 and 4 are similar views of a later stage.

6. Adult apyrene spermatozoon, drawn from a specimen fixed with osmic vapor and stained with iron hæmatoxylin.

7. Adult eupyrene spermatozoon, drawn from a specimen fixed with osmic vapor and stained with iron hæmatoxylin.

PLATE 2.

(Initial magnification 3450 diameters, reduced one-third.)

8 to 14. Various stages in the growth of the apyrene spermatoblast from the earliest appearance of the centrosomal structure until a time just before it disappears and the nuclear membrane is dissolved.

15 and 16. Two consecutive sections through a single cell showing the initiation of the changes which occur to the nucleus and the centrosome. The mitochondria are shown lying around the centrosome (fig. 15).

17 and 18. Two consecutive sections through a single spermatoblast in the same stage as the preceding one. Note that the centrosome has disappeared.

19 and 20. Two consecutive sections through a single spermatoblast showing the scattering of the chromatic masses after the dissolution of the nuclear membrane.

PLATE 3.

(Initial magnification 3450 diameters, reduced one-third.)

21, 22, and 23. Three consecutive sections through a single spermatoblast showing the appearance of groups of centrioles at the periphery of the cell.

24 and 25. Two consecutive sections through a single spermatoblast showing the completed fragmentation of the chromatic masses to form the ultimate karyomerites.

26 and 27. Two consecutive sections through a single spermatoblast showing the appearance of the centrioles on the periphery of the originally proximal half of the spermatoblast and the vesiculation of the karyomerites.

28 and 29. Two consecutive sections through a single spermatoblast, showing the grouping of the centrioles and the growth of the flagella.

30, 31, and 32. Three consecutive sections through a single spermatoblast of the same age as the preceding one, showing the rays which extend from the centrioles into the interior of the cell.

PLATE 4.

(Initial magnification 3450 diameters, reduced one-third.)

33. A section through a very young spermatosome. The centrioles have gathered at the base of the cell but have not yet divided.

34. A section through a spermatosome in the stage in which the centrioles are dividing.

35. A section through a spermatosome after the division of the centrioles showing the manner in which the axial fibers are formed.

36 and 39. Sections through two spermatosomes showing consecutive stages in the growth of the axial fibers.

37 and 38. Two consecutive sections through the bundle of axial fibers of a spermatosome in a stage corresponding to that shown in figure 39. Note the almost round base and the flattened anterior portion of the bundle and the mitochondrial apparatus surrounding the latter.

40 and 41. Sections through two spermatosomes of approximately the same age; the sections pass in planes which are at right angles to each other. Figure 41 gives a surface view of the bundle of axial fibers and the mitochondria surrounding it; note the increase in the size of the chromatic vesicles shown here.

42. A cross-section through the posterior region of a spermatosome which is a little older than the preceding ones. Note how closely the mitochondria are applied to the bundle of axial fibers.

PLATE 5.

(Initial magnification 3450 diameters, reduced one-third.)

43. A section through a spermatosome showing the further growth of the bundle of axial fibers.

44 and 45. Sections through two spermatosomes showing the bundle of axial fibers extending across the cell and the early formation of the secreted bodies; the sections pass in planes which are at right angles to each other.

46. A section through a spermatosome showing the continued growth of the bundle of axial fibers until it extends beyond the cell.

47 and 48. Sections through two spermatosomes of slightly different ages showing the splitting of the bundle of axial fibers; the sections pass in planes which are at right angles to each other.

PLATE 6.

(Initial magnification 3450 diameters, reduced one-third.)

49. A longitudinal section through a spermatosome in a well-advanced stage. The splitting of the bundle of axial fibers has been completed and the cell is partially filled with the secreted bodies. The nuclear vesicles are rapidly degenerating.

50 to 58. Consecutive cross-sections through a single spermatosome a little younger than that shown in figure 49. The sections are taken in order from the posterior to the anterior end of the spermatosome and show the modifications of the two halves of the bundle of axial fibers as they reach the cell-wall on each side.

59 and 60. Cross-sections through the posterior and anterior halves of two spermatosomes of the same age showing some of the axial fibers moving out beneath the cell-wall.

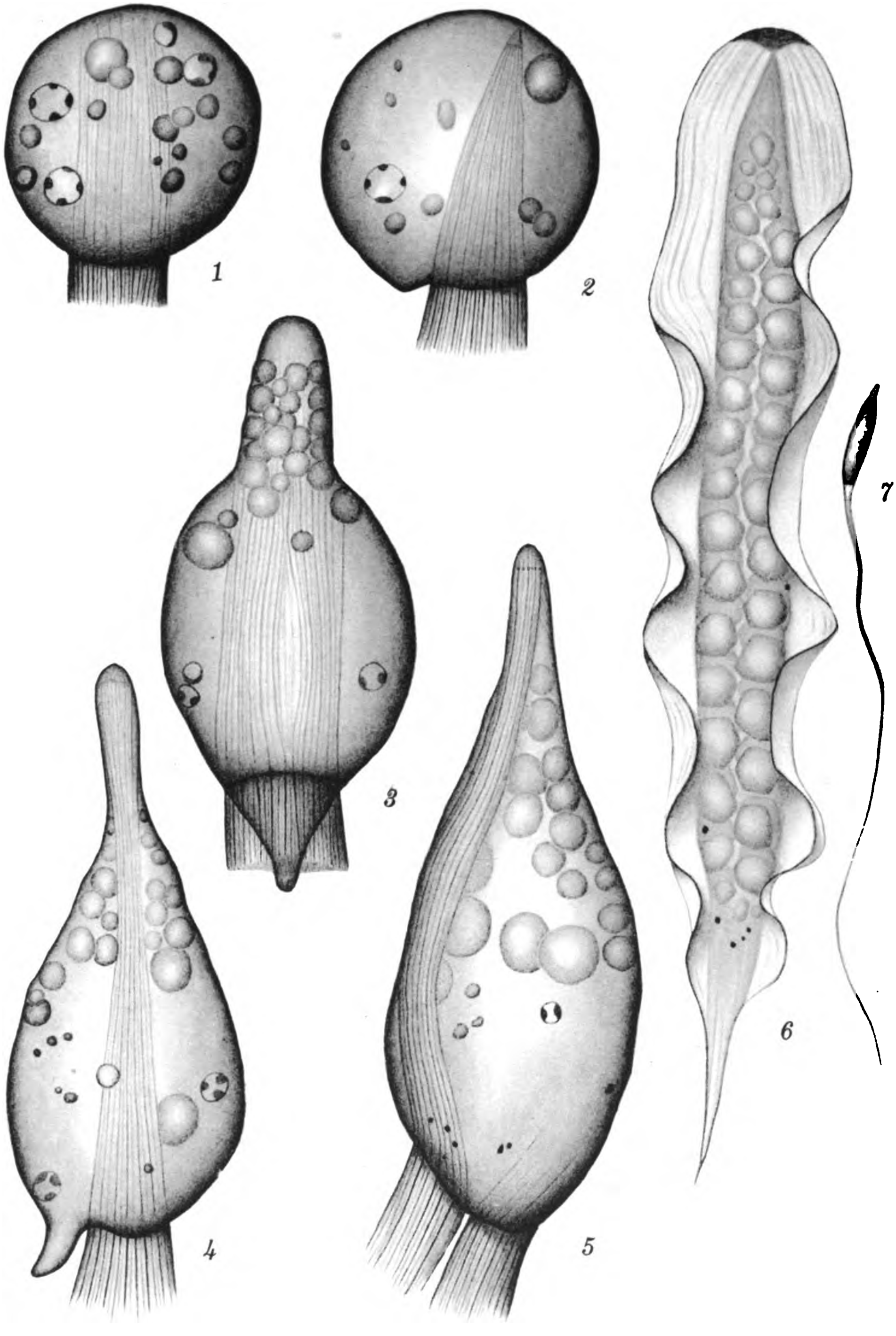
PLATE 7.

(Initial magnification 2450 diameters, reduced one-third.)

63. A longitudinal section through a spermatosome in which the halves of the bundle of axial fibers have reached the cell-wall on both sides throughout their entire length. The secretion of the albuminous bodies has not yet been completed nor has all the chromatin disappeared.

64. A longitudinal section through a nearly adult apyrene spermatozoon showing the total disappearance of the chromatin and the cell filled with the secreted bodies.

65. A longitudinal section through an apyrene spermatozoon of the same age as that shown in figure 64 giving a surface view of one of the two undulating membranes and the longitudinal fibers beneath the cell-wall.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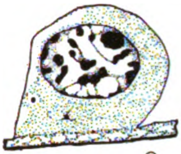
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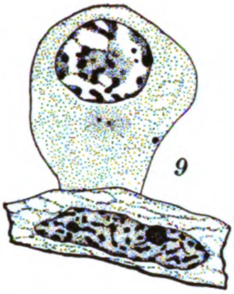
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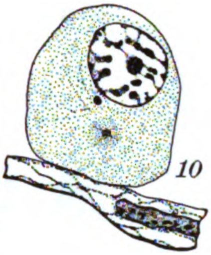
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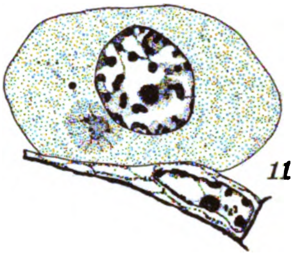
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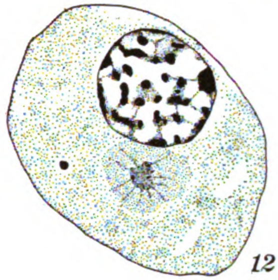
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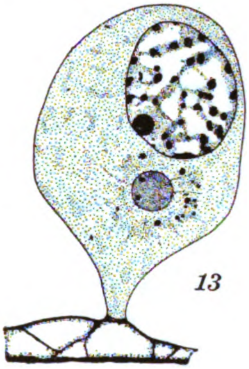
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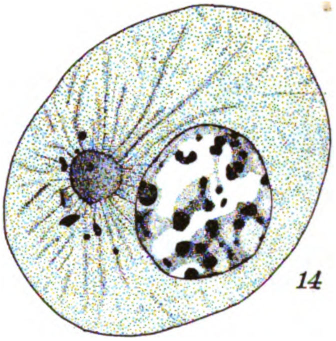
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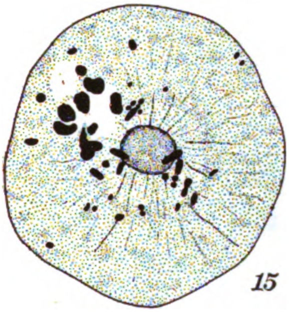
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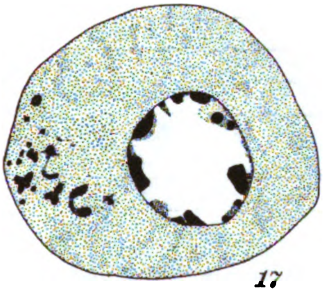
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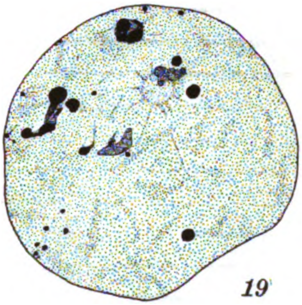
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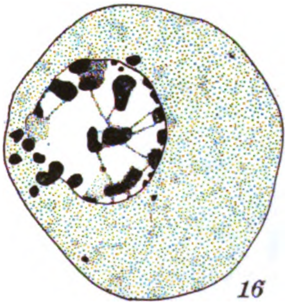
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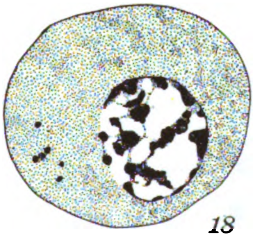
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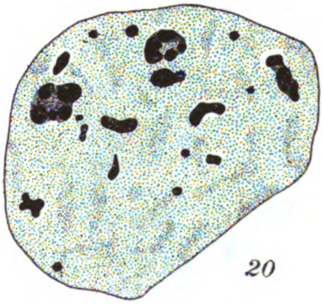
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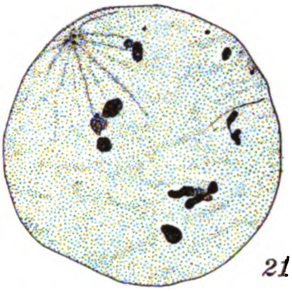

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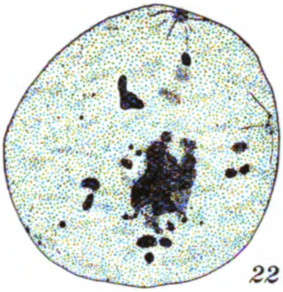

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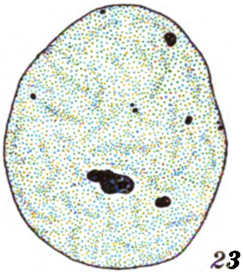

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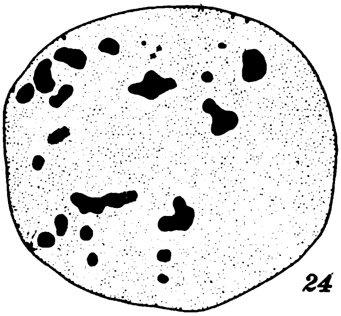

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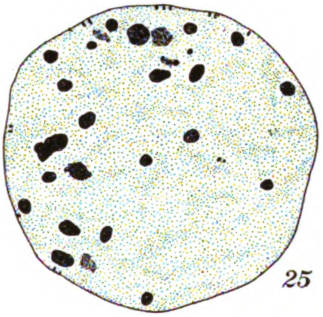

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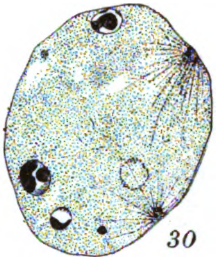

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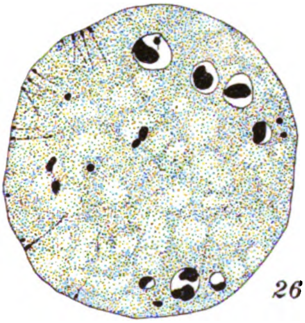

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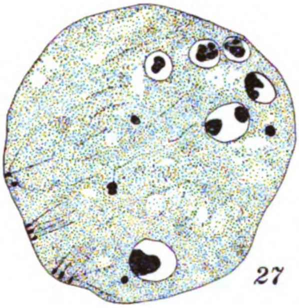

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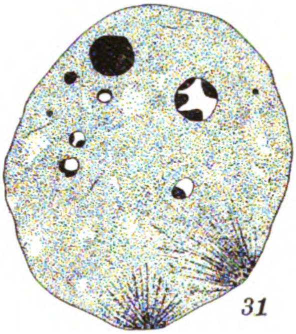

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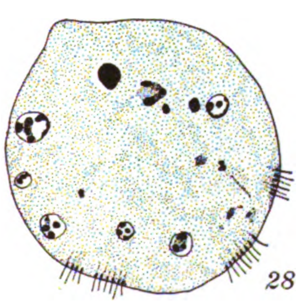

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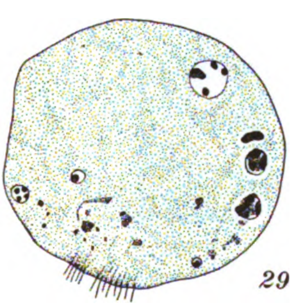

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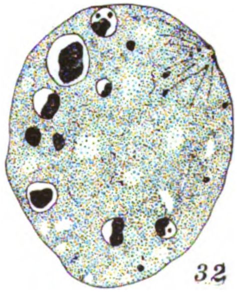

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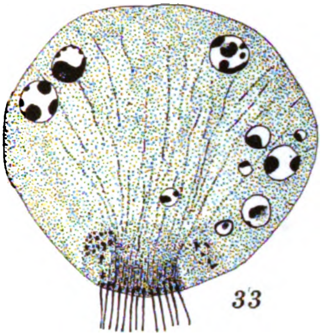
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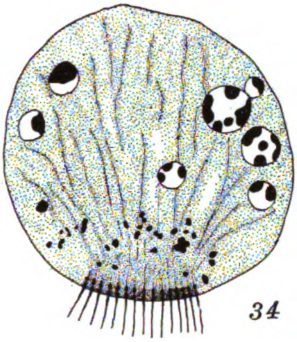
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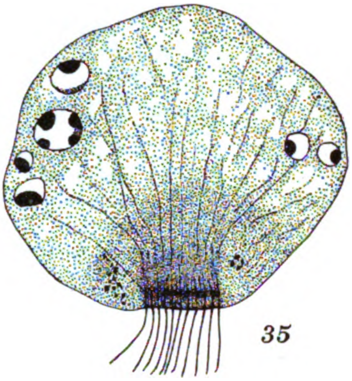
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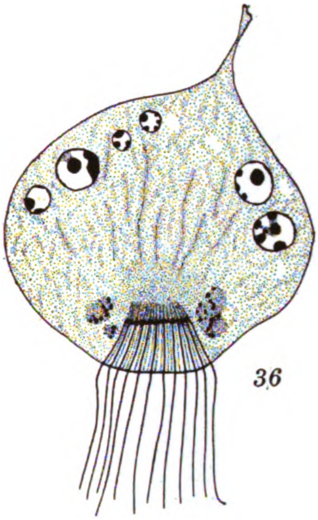
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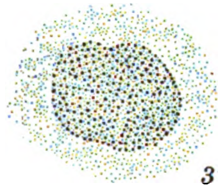
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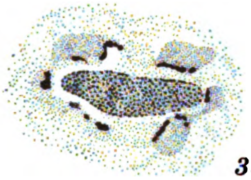
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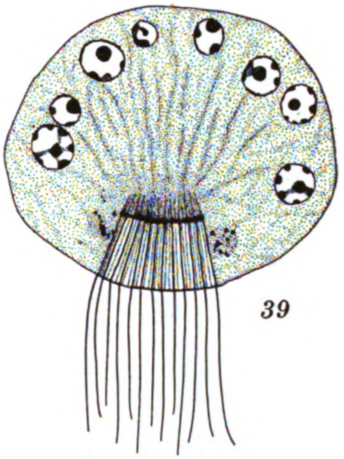
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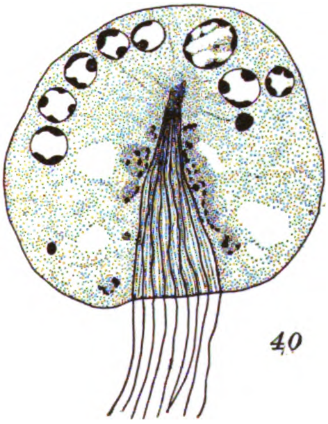
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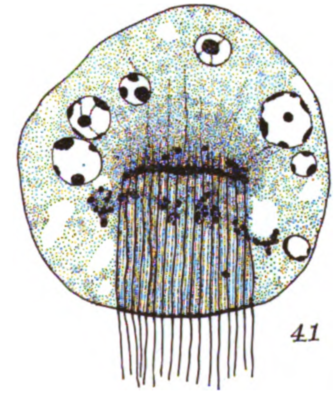
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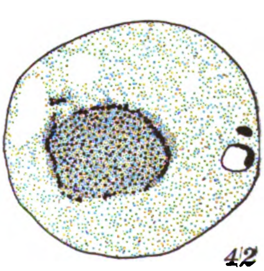
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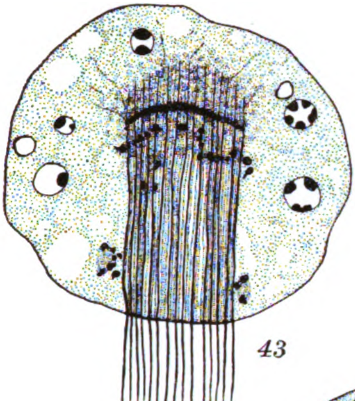

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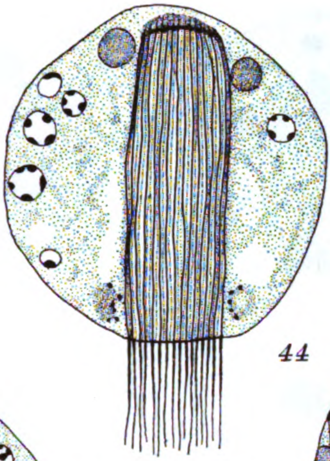

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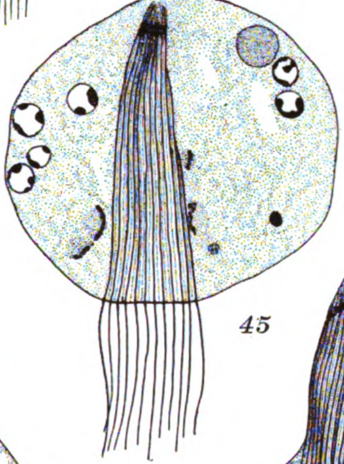

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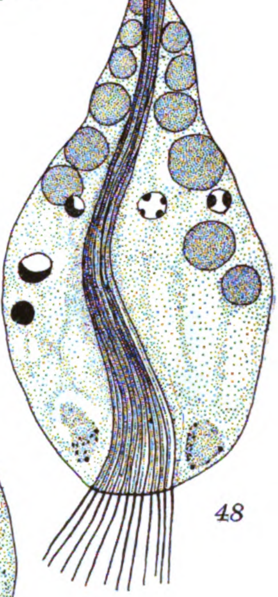

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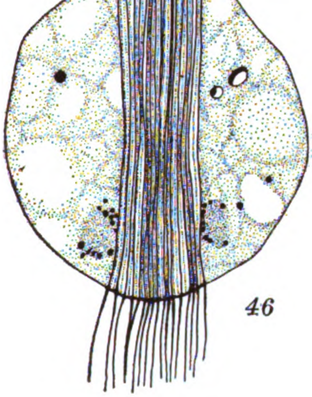

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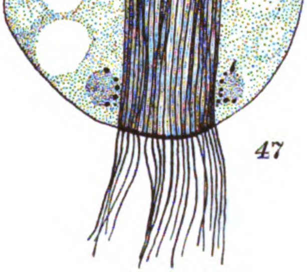

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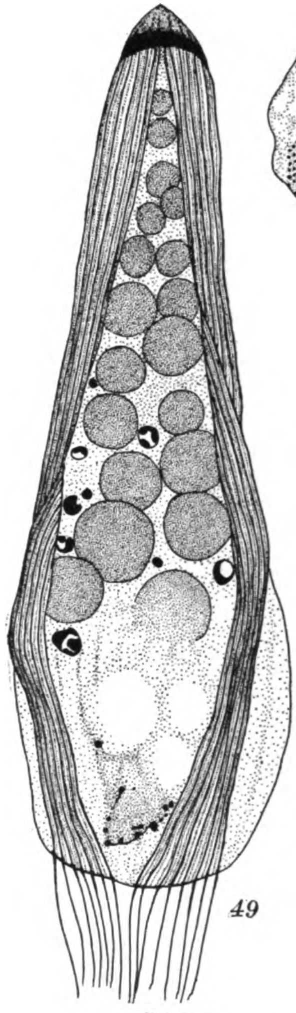
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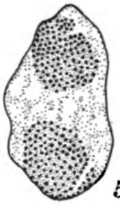
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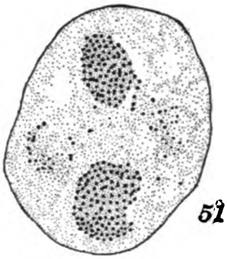
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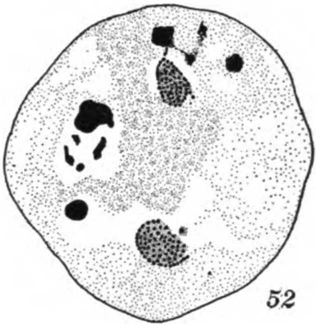
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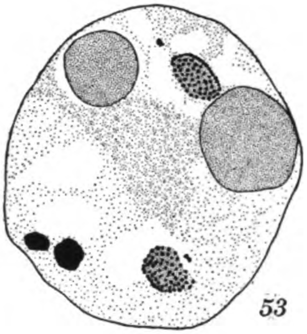
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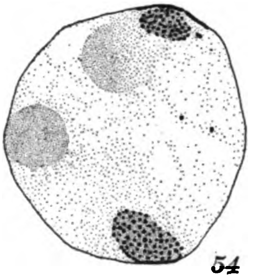
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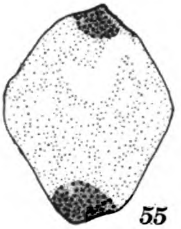
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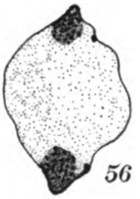
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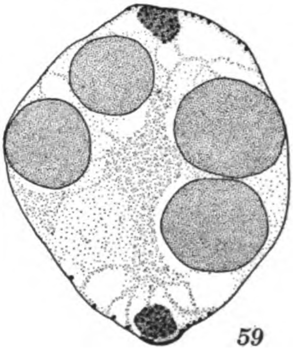
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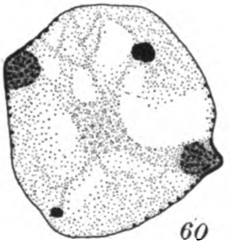
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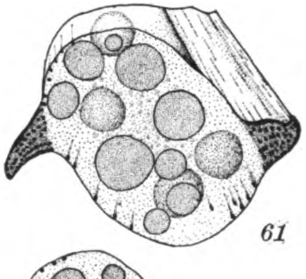
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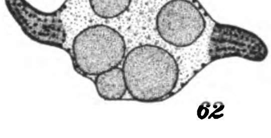
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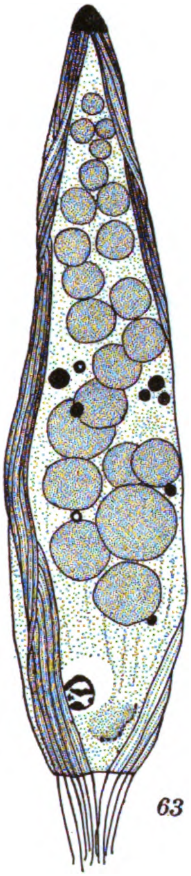

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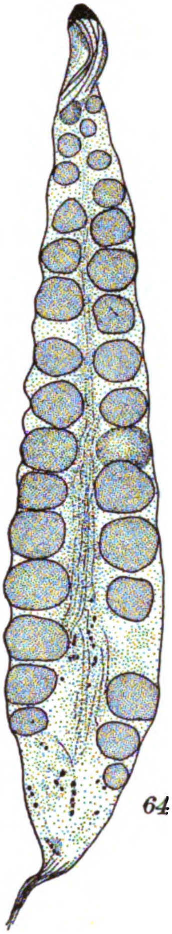

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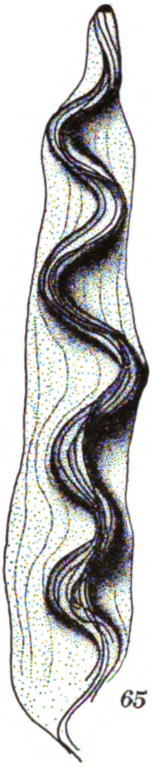

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XII.

HISTORY OF THE SPOTTED EAGLE RAY, AËTOBATUS NARINARI, TOGETHER WITH A STUDY OF ITS EXTERNAL STRUCTURES.

By E. W. GUDGER,
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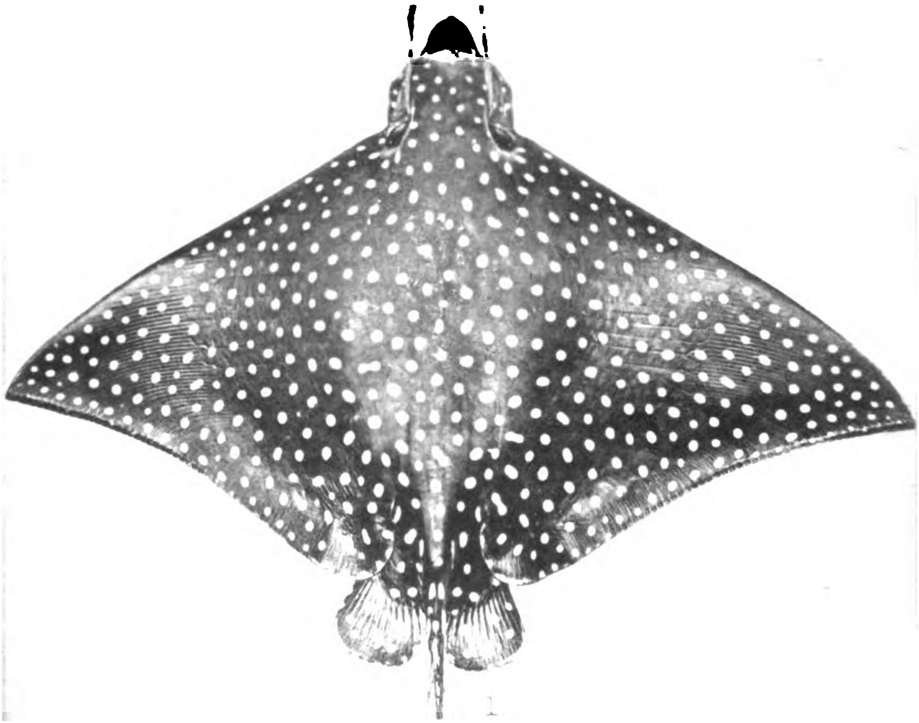
Ten plates and nineteen text-figures.

CONTENTS.

The Spotted Eagle Ray, *Aëtobatus narinari* 243
General Descriptions 244
Specific Descriptions 261
    Color and markings 261
    Tail and spines 274
    Head and snout 280
    Jaws and teeth 283
    Dorsal sensory pore system 294
Habits 296
    Feeding habits 296
    Offensive and defensive habits 298
    Breeding habits 300
Habitat 303
Economic value 305
Genera and Species of Angle-toothed Rays 306
    Aëtobatus narinari 306
        Genuine synonyms 306
        Doubtful synonyms 309
    Aëtobatus flagellum 312
    Aëtobatus guttata 313
    Aëtobatus latirostris 314
    Unestablished genera and species 315
Synonymy 317
Bibliography 319



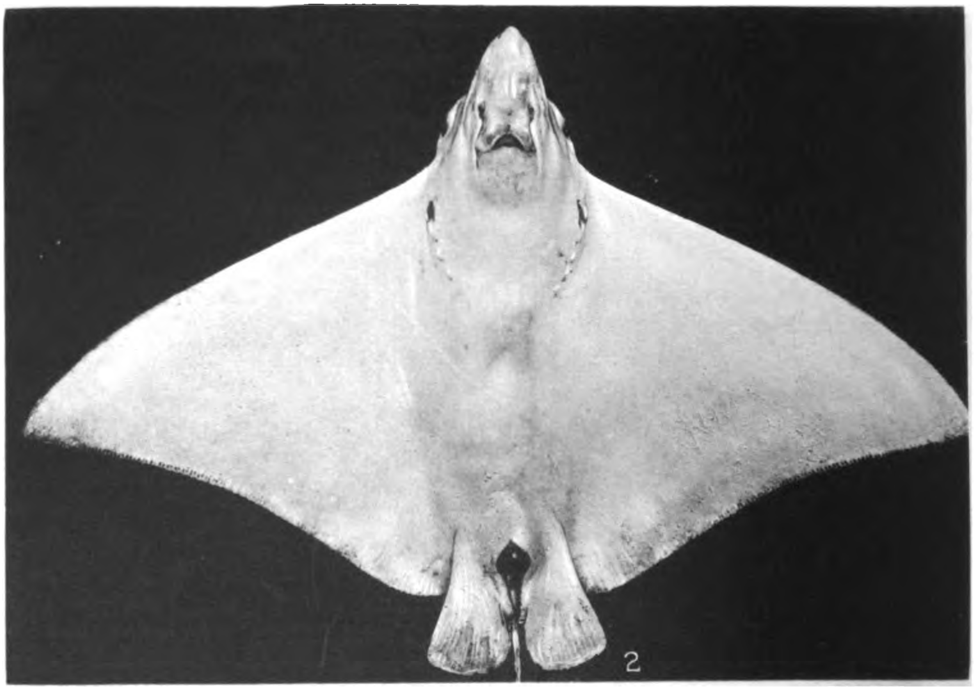


FIG. 1. Dorsal view of *Aetobatus narinari*, from Beaufort.
FIG. 2. Ventral view of *Aetobatus narinari*, from Beaufort.

HISTORY OF THE SPOTTED EAGLE RAY, AËTOBATUS NARINARI, TOGETHER WITH A STUDY OF ITS EXTERNAL STRUCTURES.

BY E. W. GUDGER.

This paper had its inception in the desire to record permanently certain observations which I was so fortunate as to make on this ray at the Laboratory of the United States Bureau of Fisheries, at Beaufort, North Carolina, in the summer of 1909; but, it was very quickly seen that the value of the paper would be greatly increased if it included a review of the better-known literature of the fish. Before this could be done, however, I again found myself at Beaufort (1910), where I was more fortunate than ever in getting specimens. Later (1913), while working at the Marine Laboratory of the Carnegie Institution of Washington at Tortugas, Florida, a number of fine specimens were obtained from Key West, making possible a comparison of Florida and North Carolina forms. Meanwhile the study of the literature proved of very great interest, and finally it was determined to make the paper include a review of all previous work on this ray. To this end neither time nor effort has been spared and it is believed that the paper justifies its title, since in it every scientific article has been reviewed and every figure of the ray reproduced (with three exceptions noted later), which so far as I know have ever been published.

In such a work, done under many difficulties and at great distances from libraries, I am under many obligations to many people, and it is a pleasure to acknowledge my indebtedness to those who have made this paper possible. To Dr. Theodore N. Gill and Dr. Barton W. Evermann I am indebted for much invaluable advice and suggestions. To the United States Bureau of Fisheries I wish to express my thanks for the fact that the facilities of its laboratory at Beaufort, North Carolina, and of its library at Washington have been freely put at my disposal. My hearty thanks are due Dr. A. G. Mayer, who not only afforded me every facility of his laboratory at Tortugas and Key West, but whose interest led him personally to procure for me three of my four Florida specimens.

THE SPOTTED EAGLE RAY, AËTOBATUS NARINARI.

Among the selachians, or fishes with exposed strap-shaped gill-slits, the order Batoidei, skates and rays, is easily the most interesting because of the very extraordinary and unusual forms assumed by these flat-bodied, disk-shaped animals. All the families of North American rays are represented along the shores of eastern North Carolina, but of them the eagle rays, myliobatids, are the most attractive. These are readily recognized by their wing-like pointed pectoral fins, their long, slender, whip-like tails armed with one or more serrated spines, and their jaws filled with large pavement teeth. Of the three North Carolina genera of the family Myliobatidæ, two forms, *Aëtobatus narinari*, the spotted eagle ray, and *Rhinoptera bonasus*, the cow-nosed ray, have been studied by the writer, while with the third and rarer form, *Myliobatis freminvillei*, he is but slightly acquainted.

Because of its shape and markings, the spotted eagle ray is easily the handsomest of these forms, and, in the whole ray order, so far as represented on the North Carolina coast, its only rival in appearance is the butterfly ray, *Pteroplatea maclura*.

GENERAL DESCRIPTIONS.

The earliest reference to *Narinari*, which the writer has found, is in an old book by Claude d'Abbeville, "Histoire de la Mission des Pères Capuchins en l'Isle de Maragnan,"[1] published at Paris in 1614. On page 245, this old monk writes as follows:

> There is here the *Narinary*, which is another flat-fish very similar also to the Raye. It is six feet long and as many wide, and has a tail about a fathom long, in the middle of which there is, moreover, a spine [as in the preceding ray], but about a full foot in length and very dangerous. This ray is all striped with black and white.

This is very indefinite, since none of the distinctive characters of the fish are given, but the reference is probably to the ray under study. Sloane (1797), from whom the reference came, thinks so. The name, *Narinary* or *Narinari*, is Brazilian, and seems to have been the common designation of this fish. The description of the lines is very interesting, since only one of the several published figures of the fish, that by Jordan and Evermann (1900), shows these lines (text-fig. 3), and but one author, Smith (1907), describes them.

The next reference is found in Jan de Laet's "Novus orbis, seu descriptiones Indiæ Occidentalis," published at Leyden in 1633. In book XVI, chapter 14, page 616, de Laet quotes from Abbeville that *Narrinnari* (also spelled in the margin *Narinnary*) is a species of ray having a tail shorter, but with spines longer (than certain forms previously referred to), and having the whole body marked with black and white lines. Beyond this brief statement he gives no further description, and it is pretty certain that he never saw the fish.

In Purchas (1625), "His Pilgrims," vol. VII, chapter I, is a "Treatise on Brasil, written by a Portugal which had long lived there." On p. 1313 is an obscure reference to a fish which "hath in its mouth two stones as broad as the hand, exceedingly strong, with which they break the Wilkes whereon they feed." And in the next paragraph "these Rayes some have in their mouths two bones, and break with them the Wilkes." These statements

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[1] The island of Maragnan, or Maranham, as the Portuguese wrote it, is the present Maranhao on the northern coast of Brazil.

Sloane (1725), who first notes them, thinks refer to *Narinari*, and it is certain that they refer to the jaw structures of a Myliobatid, or mill-toothed ray.

In 1638, in the capacity of astronomer and naturalist, George Marcgrave accompanied the Dutch expedition to Brazil under the leadership of Maurice of Nassau, who was governor-general of the Dutch conquests in that country from 1637 to 1644. Marcgrave remained in Brazil and northeastern South America for several years. With him went William Piso, who was physician to Count Maurice. While these two men accompanied the expedition in their professional capacities as physician and astronomer, it is certain that as naturalists they had the active aid and cooperation of Count Maurice, as will be indicated later.

Piso's scientific work on this expedition was done wholly from a medical standpoint and is embraced in four books, comprising 132 pages with 104 figures, and bearing the title "De Medicina Brasiliensi." Marcgrave's natural history work is embodied in eight books covering 303 pages, illustrated by 429 figures, and entitled "Historiæ Rerum Naturalium Brasiliæ." Books I, II, and III treat of plants, book IV of fishes, V of birds, VI of quadrupeds and serpents, VII of insects, VIII of the land and its inhabitants, and finally there is an appendix on the Tapuyis and Chilians.

The joint work of Marcgrave and Piso, edited by de Laet, was published in folio at Leyden and Amsterdam in 1648, under the title "Historia Naturalis Brasiliæ." Although Marcgrave's part of this work is more than twice as great as Piso's, he being dead, Piso's contribution fills the first part of the volume and on the title page Piso's name precedes that of Marcgrave.

That part of Marcgrave's work dealing with the fishes of Brazil is the first study ever made of the fish fauna of a region outside of the Mediterranean Sea. He describes 105 species and gives spirited figures of 86 of them, all new to science. To book IV of Marcgrave's "Natural History of Brazil," the student of American ichthyology must go back for the original descriptions and figures of a large number of our fishes. So the present writer has done for the spotted sting ray and the toadfish studied by him. This "Natural History of Brazil," at the time of its publication, was by far the most scientific and comprehensive work of its kind given to the world and it was more than a hundred years before a work of equal importance appeared. Too much praise can not be given Marcgrave and his patron and friend, Prince Maurice, for what even in our day would be a great undertaking.

The Royal Library of Berlin contains two collections of paintings, in folio, of the natural history objects of Brazil. These paintings number 1,460 in all. The larger collection is in oils and bears title as follows "Theatri rerum naturalium Brasiliæ. (Icones in 4 Bänden), Libri Picturati A 32–35." The smaller collection is in water colors and is entitled "Brasilianische Naturgegenstände (Collectio rerum naturalium Brasiliæ in 2 Bänden). Libri picturati A 36–37." In the present writer's biographical sketch of Marcgrave (Gudger, 1912) it is clearly proved that the water-color figures, from which Marcgrave's "Natural History of Brazil" was illustrated, were painted by Marcgrave himself. It seems equally clear that the oil paintings were by a Dutch painter named Franz Poste, who accompanied Count Maurice to Brazil. The whole matter of the paintings is discussed in full in the sketch cited above. These paintings will be referred to later.

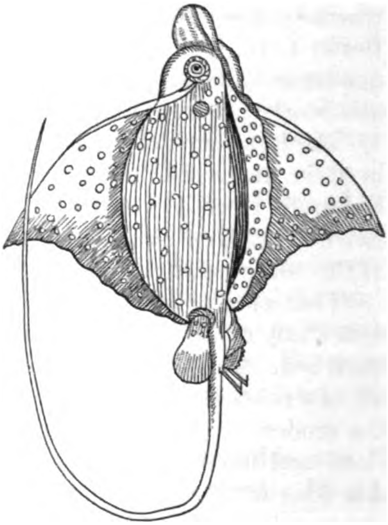
Some time during the years 1638–44 Marcgrave discovered, figured, and described from Brazilian waters the spotted eagle ray, and inasmuch as his original description and figure possess great historical as well as scientific value, since they show what excellent work was done in a wild and barbarous country nearly 275 years ago, by a man working under great disadvantages but possessed of the true scientific spirit, they are herewith reproduced from pages 175 and 176 of the above-mentioned work by Piso and Marcgrave (1648) (text-fig. 1):

Of the several species of fish called "Narinari" by the Brazilians, the one which we have described here is "*Narinari pinima.*" It is called "*Raja*" by the Portuguese, and "*Pylsteerte*" or "*Siecle*" by the Dutch. It is a "*Marina pastinaca.*"

Its body is large, broad, almost triangular in shape, extending out on both sides into very broad triangular wings, which are fleshy in their make-up. Near the tail it has two fins about the size of one's hand, rounded in outline and of equal length. Its head, which is thick, compressed, and furrowed in the middle, is about as large as that of a good-sized pig.

The mouth rounded underneath is triangular, compressed a little, and terminates in a snout. The opening of the mouth is on the ventral surface, 5 inches from the end of the snout. The mouth is 2½ inches wide, toothless, but having in the place of teeth a lower jaw in the shape of a tongue. This is 4 inches long, 1½ inches wide, and reaches to the external opening of the mouth. Likewise, there is an upper jaw placed crosswise, 2 inches long and as many wide.

The lower jaw consists of 17 hard white bones having the shape of the letter U and firmly joined to the membranes. Underneath there lie 17 other bones, one under each, of spongy appearance but not so hard. The upper jaw consists of 14 bones, shaped like the letter J and also joined together by membranes. Likewise 14 other bones lie above these. Moreover the two jaws are joined to the other bones of the head by membranes [cartilages?].



TEXT-FIG. 1.—*Narinari*, from Marcgrave.

The cavity of the skull, wherein the brain lies, is about 6 inches long and hardly 2 wide. The snout is wholly cartilaginous. The fish has two small eyes about the size of a *nummus misnicus*. Behind these eyes, on each side, is a large breathing-hole capable of holding an apple of ordinary size. Within these holes the leaves of the gills lie hidden. On the lower side at the [hinder] end of the head are five oblong incisions.

The whole upper surface of the body is of a steel (*ferreus*) color with white spots the size of a *nummus misnicus* scattered over it, while the under part is entirely white. The skin is everywhere smooth and without scales.

The length of the body from the end of the snout to the root of the tail is 1½ feet; the width between the extremities of the triangular wings is 3 feet 10 inches. The length of the fins near the tail is 7 inches, the width 4. The length of the head is 10 inches, the width 7, and it is 1½ feet thick. The tail is 4 feet 3 inches long and its thickness at the beginning is 5 inches, but it gradually becomes thinner. A little behind the beginning of the tail, there is a small, short fin, a little more than an inch long; and just behind this, standing erect, are two little hooks, curved like fish-hooks and 3 inches long. Its flesh has a good flavor and is sufficient to feed 40 men.

This description needs little or no comment here. It is excellent and would enable one to identify the fish to-day. The statement that the head of the ray is 1½ feet thick, is, inasmuch as the length of the animal is only 18 inches, evidently an error (either editorial or typographical, since the work was published four years after Marcgrave's death). So, likewise, is the statement that the tail is 5 inches thick at the root.

Text-figure 1 is Marcgrave's wood-cut made from the oil painting in the collection referred to. The figure in Marcgrave's text has been colored by hand in one copy of the work found in the Library of Congress, but in the other copy, as in that owned by the Bureau of Fisheries and in the copy belonging to the present writer, the figures are uncolored. The upper surface in the colored figure is a dark steel-blue (*ferreus* Marcgrave calls it) dotted with white or bluish-white spots. Attention should here be called to the discrepancy between the description and the figure in the matter of spines. The former has them curved like fish-hooks, but the figure has them straight, with one large barb on each. As a matter of fact, they are of the ordinary ray type, straight, flat, and multibarbed on the edges.

Figure 3, plate II, is a photograph of the water-color painting to which reference has been made. When it is recalled that this painting was made some 275 years ago, on the wild and inhospitable coast of Brazil, one hardly knows whether to marvel most at the accuracy of the work or the excellency of its preservation. If this figure be compared with text-figure 1, it will be seen how poorly the wood engraver has copied the original painting. Concerning these figures, see Gudger (1912, pages 265–272).[1] The marginal notes on the original painting are presumably those of Count Johann Moritz. Figure 4, plate II, is a photograph of the oil painting in the larger collection described above. The deficiencies of this latter figure, when compared with the former or with my photograph (fig. 1, plate I), are so marked as to call for no comment here.

The older writers seem to have contented themselves with merely quoting Marcgrave and copying his figures. So did Piso himself, in his folio volume entitled "De Indiæ Utriusque re Naturali et Medica," etc., Amsterdam, 1658, though he abridged Marcgrave's original description somewhat. However, he does one good deed in giving us a clue to the meaning of the Brazilian word *Narinari*. He tells us that the tail is armed with two stings "approaching in form the figure of an arrowhead, and therefore these fishes are properly called by us *Pylstaert*, and by the Brazilians *Narinari*." Now since the Dutch word *Pylstaert* means both a water bird and a sting ray, we might think that the allusion was either to the wing-like pectorals or to the spined tail, had not Piso made it clear that the latter is meant. It seems then that the word *Narinari* means sting ray (see Martius, 1867).]

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[1] For these photographs and for much information about these drawings, I am indebted to the courtesy of Dr. Perlbach, of the Royal Library of Berlin.

Willughby, in his "De Historia Piscium," published after his death by Ray at Oxford in 1686, reproduces both figure and text with great exactness. The fish, however, is represented as if it were lying on its left and were drawn from the right side. The description and figure are credited to Marcgrave.

The next reference to this interesting fish is to be found in Jonston's "Historiæ Naturalis de Piscibus et Cetis." This work passed through several editions. The first, according to Walbaum, was published at Frankfort in 1649. Three editions are dated at Amsterdam in 1657, 1660, and 1718. In the Latin version in the Library of Congress, bearing date of 1767, Jonston reproduces Marcgrave's figure, but reverses right and left sides, as does Willughby, and takes out some of the crudities of the drawing, and quotes Marcgrave's description almost verbatim, giving him credit.

Henry Ruysch, in 1718, published his "Theatrum Universale Omnium Animalium, Piscium," etc., which seems to be a compilation of the works of various writers from Aristotle to Marcgrave and Jonston, but mainly of the latter. Ruysch has rearranged Marcgrave's description and figures, probably following Jonston therein. He has copied Marcgrave's description of *Narinari* in the minutest detail, but without a word of reference or acknowledgment. He reproduces Marcgrave's figure, but has improved it, especially in the shading.

In 1697 Hans Sloane figured and described in volume XIX of the "Philosophical Transactions" the jaws of a *Pastinaca marina* from Jamaica, which he says is identical with Marcgrave's *Narinari*. In his brief description of the fish he says that it is "smooth, blue; covered with white spots." Later (1725), in volume II of his Natural History of Jamaica, he describes this fish more fully.

> This [whip ray] was about two foot over from corner to corner, and all blue even the flesh itself with white spots on it, the under side or Belly was white, as in others of this kind, the tail was six foot long, black, small and smooth, of which are made whips, whence the name Whip-Ray, beyond the Pinna at the end of the body or in the beginning of the tail lie one, two or three inch and a half long flat streight bones or Radij; they are white, serrated with Teeth on both Sides like a saw, made so as an arrow that's barbed, to enter the flesh easily but not to come out without tearing it, they lie one on another on the upper part of the tail, where there is a hollow or cavity made to receive them like a sheath, that they may swim with less Impediment and only use them on Occasion.

It is probable that Patrick Browne, in his "Civil and Natural History of Jamaica" (1756), refers to our ray when, on page 459, he describes a whip-ray thus: "Middle parts bluish mixed, tongue long, with a barbed spine on the finned tail." Ray (1713), who it will be remembered edited Willughby's manuscript, in his "Synopsis Methodica Piscium," quotes Sloane and Marcgrave, but adds nothing new concerning our ray.

I find no other reference to *Aëtobatus narinari* until 1790. Excepting Abbeville, Marcgrave, and Sloane, and possibly Piso, none of the older writers above quoted seems to have ever seen the fish; they were all compilers. However, in the above-named year, a Swede, Bengt Anders Euphrasen, described and figured a specimen of this ray which had been taken at the island of St. Bartholomew, one of the West Indies at that time belonging to Sweden. He calls this fish *Raja narinari*, thus fixing the specific name. He refers to Willughby, whose description and figure he criticizes, seemingly in ignorance of Marcgrave, from whom Willughby copied both figure and description.

Euphrasen's figure, which was drawn in Sweden from a preserved specimen, is herewith reproduced (fig. 5, plate III), but modified slightly in one unessential detail. In the original figure, the long tail hangs down straight save for a fish-hook-like curve at the tip; in the reproduction, for the sake of economy in space, it is curled to the left as shown; the length, however, is correct. Euphrasen describes his specimen as follows:

> Head prominent, compressed, in form like a toad's, on top 2 pits placed transversely. Mouth ventral as in its kind, transverse, teeth few and closely crowded. Eyes lateral, of medium size when compared with the head. Ears (*aures*) above the neck, or at the base of the head, with apertures round, and a little larger than the eyes. Spiracles 5, under the throat on both sides as in its kind.
>
> Body flat, very broad, ending laterally in acute angles, the margin feathered (*pinnis*). Above brown[1] (blue) with round white spots, in diameter about the size of the thumb, scattered about; beneath white. Anal fins one on each side at the base of the long tail, reaching to the base of the spine on the anterior part of the tail. Dorsal fin small, subtriangular, situated on the base of the tail. Spines 2, oblique, dorsally placed behind the fin, compressed, retrorsally barbed, the posterior double the length of the anterior. Tail whiplash-like, compressed, attenuate from base to apex, without fin, 3 times longer than body.

In Walbaum's edition of Artedi's "Bibliotheca Ichthyologica," part III (1792), the former quotes the name *Raja narinari* from Euphrasen's paper above named, refers to Willughby and Marcgrave, and gives this brief description: "Body smooth, steel colored above, with numerous white spots."

About this time, *i. e.*, the close of the seventeenth and the beginning of the eighteenth century, a number of ichthyologists include *narinari* in their lists of rays without adding anything to our knowledge of the fish and some of them fall into the grievous error of applying the name to rays which are not even eagle rays much less Aëtobatids. Bloch in volume III of his "Ichthyologie" (1786) appends the name *narinari* to an ordinary sting ray with a long tail, and which he expressly states has *many* rows of teeth in its jaws. Other cases might be cited, but to no purpose.

In the closing years of his life, Bloch had outlined and made some progress on a great ichthyological synopsis after the plan of Linnaeus's "Systema Naturæ." After his death, this important work was brought to completion by J. G. Schneider (Bloch and Schneider, 1801). Beginning on page 361 we find the description of *Raja narinari*. This I believe to be the work of Schneider for two reasons; first because in Bloch's other works there is nowhere found any description of *A. narinari*, and in the second place because the account under discussion is written in the first person.

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[1] Euphrasen uses the word *chalybeum* from chalybs, meaning steel or iron, but as his figure is a sepia-brown it may be that the color of his fish was brown.

The description of this ray is interesting not because it contains anything new or unusual or erroneous, but because it is a curious jumble of Marcgrave, Willughby, Euphrasen, J. R. Foster (MS. "Descriptiones Animalium"), Prince Maurice of Nassau, and Hans Sloane ("History of Jamaica"). However, Schneider examined several "Swedish specimens," and among them probably Euphrasen's, while he also had several sets of jaws and a dried specimen cut in half.

Omitting those parts of Schneider's description, which will be taken up in the special sections, his general account is now given. He says that the head is compressed and prominent; above the apex there may be noted holes (spiracles) placed transversely; the mouth is inferior and is filled with a few teeth crowded together; the laterally placed eyes are of medium size in round orbits but little larger than themselves; the body is broad and flat, the lateral angles are acute, the emarginate fins are steel-colored above with round white spots the size of the thumb, and the tail is three times longer than the body. Its habitat is given as the American Ocean, especially around the Caribbean Islands.

In 1803, the East India Company published in London, in two sumptuous volumes, "Descriptions and Figures of Two Hundred Fishes Collected at Vizagatapam on the Coast of Coromandel" by Patrick Russell, a physician in its employ. On page 5, there is described a ray called by the natives *eel tenkee* and by Russell the *ocellated raja*. Figure 6, plate III, is a reproduction of plate VIII of the above work, showing this fish. That this is *Aëtobatus narinari*, inspection of the figure and study of the description make clear. The general outline of the fish, the pointed pectorals with fimbriated hinder edges, the white spots profusely covering the body back of the shoulder region, the projecting head with its pointed snout, the relative position of the eyes and spiracles, the rounded ventrals with the dorsal placed well between them on the root of the tail, all spell *narinari*. When one reads that the tail is long and whip-like, and that the jaws are dissimilar, "the lower arched, narrow, and projecting beyond the wider immovable upper jaw," one is quite sure that it is *narinari* despite the absence of spots on the anterior dorsal region and the fact that both jaws are described as devoid of teeth. These points will be considered later.

Blainville, in the Journal de Physique, tome 83, for 1816, has a systematic paper in which he establishes the genus *Aëtobatus*, assigns the generic characters, and notes ten species, of which *narinari* is one. Later, Blainville (1828) changed the generic name to *Aëtobatis*, though a careful search through his paper fails to reveal any reason therefor. His characters for the genus are as follows:

> Body together with the pectoral fins in the shape of a bird with extended wings; head free and provided with a simple appendage in front; eyes lateral; teeth large, smooth, polygonal, united into two plates, one lingual, the other palatine; pectoral fins pointed, anterior border convex, posterior concave; pelvic fins as in the sting rays, only one fin above on the root of a tail often very long, flagellate, armed with one or two spines.

This, it should be noted, is a statement of the characters of the genus only; *narinari* is not named and no specific description is given save for *Raja aquila* of European waters.

In the celebrated voyage around the world of the corvette *Uranie*, a spotted sting ray was taken at Guam. The zoology of this voyage was written up by Quoy and Gaimard (1824), the ship's surgeons. They described their ray as having an elongated snout, while its general color was a light brown sprinkled with round spots of cerulean blue, and its tail was armed with five very long, barbed spines. Their description is very imperfect and their drawing "made on the spot" was lost. They were much impressed by the fact that this ray had five spines on the tail and refer to it as such an extraordinary matter as to make the tail worthy of deposit in the Museum (of Paris?), and from this phenomenon gave to the ray the name *Raja quinqueaculata*, the 5-spined ray. A photographic reproduction of their fine figure of the spines is given in figure 7, plate IV, of this paper. Below in the same figure is a photograph of a portion of the tail with four stings of *A. narinari* from Beaufort. In the section dealing with the tail and its spines, a careful comparison of the two tails will be made.

In 1835, Ruppell described an eagle ray from the Red Sea under the name *Myliobatis eeltenkee*. However, he has doubts about the identity, for he says that he is not able to say whether it is different from Linne's "form called *Raja narinari* after Marcgrave's description."[1] In his synonymy, Ruppell says his fish is identical with Russell's *eel tenkee* which Gunther says, and which we have just seen, is *Aëtobatis narinari*. That it is *A. narinari* can not be doubted when one reads of the long, pointed pectorals with fine notchings on the posterior edges (found on the ventrals also) the white spots covering the dorsal surface; the elevated head with lateral eyes; the long, pointed snout, and the mouth with its teeth in a single row in each jaw, the lower being angled outwardly. Some doubtful points are seven teeth only in each jaw (these will be considered at length later), the dark olive color above, and the flabelliform tail.

Despite the fact that Swainson (1838–1839) spent some months on the northern coast of Brazil in 1816–1817, there is no evidence that he ever saw our ray. He quotes Russell, and also Müller and Henle, whose great work was then appearing in parts, but adds nothing to our knowledge.

In 1841 the celebrated Johannes Müller, with the cooperation of Jacob Henle, published at Berlin in folio their "Systematische Beschreibung der Plagiostomen." In it they definitely established the form *Aëtobatis narinari* based upon the examination of twelve specimens from Brazil, the Indies, and the Red Sea, six being in alcohol and six dry. The congeneric *Raja flagellum* of Bloch and Schneider, which it must be remembered is declared to be identical with *A. narinari* by Günther and by Jordan and Evermann, is based on the examination of 19 specimens and one head.

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[1] In none of the several editions of Linne's "Systema Naturæ," which I have examined, is there any record of *narinari*.

They describe the upper surfaces of their specimens of *A. narinari* as brown with round, white, regularly scattered spots which are lacking only on the head, and add that the number of the spots is often small. Both my photographs and Duméril's figure (reproduced as fig. 8, plate IV, of this paper and referred to elsewhere), show that the head does not lack for spots and that the number on the body is great. Curiously enough, however, Jordan and Evermann's figure (text-fig. 3) shows very few spots in the cephalic region. The fish from which it was drawn, however, like the twelve examined by Müller and Henle, was a preserved specimen.

In the years 1772–1774, Johann Rheinold Forster collected fishes in the southern seas and later described his collection. His manuscript, however, remained unpublished, though noted by Bloch and Schneider (1801), until it was edited by Lichtenstein and published at Berlin in 1844. He collected both in Brazilian waters and in the South Pacific, and described and figured a fish which he called *Raja edentula*, but which is easily recognizable as *Aëtobatus narinari*. According to his description, this ray is lead or steel colored above with many round white spots, white below, and everywhere smooth and bare; the head, slenderer than the body, terminates in a triangular blunt snout, cartilaginous in structure, and having the edges rolled up. The mouth is typically that of *narinari;* the crescent-shaped lower teeth form a spatula projecting beyond the shorter and broader upper jaw. The eyes are lateral, prominent, and rather far back and have vertical pupils.[1] Behind the eyes are the spiracles, connected with each other and with the mouth. The tail is three times as long as the body, slender, cylindrical, pinnate (dorsal?), black, and armed with two serrate spines. Pectoral and ventral fins are crenate behind. This is an admirable description of *A. narinari*, but unfortunately accompanied by no figure. Lichtenstein does not state why the figures, made to accompany the text, were not published.

Cantor, in his "Catalogue of Malayan Fishes" (1849), describes an East Indian Ray, *Stoasodon narinari*, which seems to be identical with our ray. His description need not detain us here longer than to note that his fish is greenish-olive or greenish-gray above, while the greenish-white spots edged with black are found on all parts of the dorsum save the head and anterior margin of the pectorals. These points will be taken up later.

Bleeker (1852) declares that *A. narinari* is common throughout the East Indies. His description of this fish does not differ from that given by other authors save as to the color of the body and the structure of the jaws, consideration of which points will be entered into later. Although he gives no figure of his fish it is undoubtedly *A. narinari.*

The most elegant figure of an eagle ray that has ever been published is that of August Duméril found in tome 10 of the "Archives du Museum d'Histoire Naturelle" for 1858–61. Figure 8, plate IV, is a photographic reproduction of plate 20 from the above volume. *A* is *A. latirostris* from the region of the Gaboon (river), *B* (lower left corner) is a head of *A. nari-*

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[1] This is the earliest note made of this peculiarity.

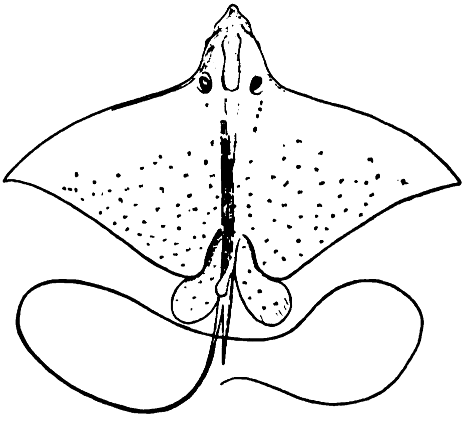
nari, *C* (lower right corner) is *A. fouet*, (*A. flagellum?*). One observes at once the larger spots of *A. latirostris*, and the fact that they are few in the cephalic region and rather widely scattered over the other parts of the body, while in *A. narinari* they are much smaller, thickly scattered, so much so on the head as to appear concentrated. Worthy of note is the fact that the congeneric form *A. fouet* is entirely devoid of spots. These differences will be discussed further in the section dealing with the species.

In 1865, Day in his "Fishes of Malabar" and Duméril in his "Histoire Naturelle des Poissons" both described *Aëtobatis narinari*, but as the distinctive points in their description have to do with the color and with the structure of the jaws, both of which subjects will be discussed fully later, and as neither published plates, further attention will not be given them here.

In the eighth volume of his "Catalogue of Fishes in the British Museum," Günther (1870) gives a description of *Aëtobatis narinari*, which, since it is based on a careful examination of no less than ten specimens (mainly from oriental waters), is possibly the most authoritative we have had since Müller and Henle. In general he agrees with the writers cited above, but gives some interesting variations in color and teeth which will be considered at some length later.

Klunzinger (1871) gives a very careful and exact description of *Aëtobatis narinari* from the Red Sea. This does not differ materially from Ruppell's earlier account (1835), but goes into much greater detail. It is interesting to note that both writers record the fact that the spiracle is broader than long; Ruppell says that it is pear-shaped. The points of chief interest in Klunzinger's description have to do with teeth, tail and spine, and color, all of which will be dealt with later in their appropriate sections.

Day (1878), in his "Fishes of India," gives a description of *Aëtobatis narinari* based evidently upon a number of specimens from Indian waters. These, however, were probably preserved specimens, since he speaks of the tail as triangular as far as the spine and compressed beyond that, structures much plainer in preserved than in fresh specimens. His figure was certainly made from a preserved fish, since it is much shrunken in the head region. The spine is disproportionally large and the dorsum is entirely devoid of spots despite the fact that in his description the body is noted as covered behind the head with dirty white or bluish spots. This figure is hardly to be recognized as *A. narinari* and it has not seemed necessary to reproduce it here.



TEXT-FIG. 2.—*A. narinari*, after Gunther, 1880.

In 1880, Gunther published his "Introduction to the Study of Fishes," but therein adds little to our knowledge of this ray. His figure (text-fig. 2) is a mere outline drawing, but is one of the best for general appearance which we have. Judged by my specimens, the ventrals are too long and too rounded, the spine too large, the depression over the brain too accentuated, while the absence of spots from the anterior part of the body is especially noticeable. The snout is good and the eyes, it should be noted, are almost invisible, as in the living fish. The same figure is given in this author's (1886) article on Rays in the ninth edition of the Encyclopædia Britannica.

After giving the general characters of the family *Myliobatidæ* and of the genus *Aëtobatus*, Jordan and Evermann, in their great work "The Fishes of North and Middle America" (1896), thus describe *Aëtobatus narinari:*

> Disk twice as broad as long, its anterior borders a little convex, posterior concave, outer angles pointed. Cephalic fin about one-third broader than long. Teeth of lower jaw straight or more or less angularly bent. Tail three or four times length of disk. Brown with small round pale spots (Duméril). Tropical seas, north on the Atlantic coast to Virginia: not very common on our shores. Narinari, a Brazilian name.

Their figures, both dorsal and ventral, are herein reproduced as text-figures 3 and 4. They were made from a preserved (alcoholic?) specimen taken near Cedar Keys, Florida, and deposited in the United States National Museum. Beyond calling the reader's attention to the parallel dark markings on the dorsum, which are not mentioned in the text, criticism is deferred until later.

The writer has elsewhere (Gudger, 1910) published measurements and brief descriptions of two spotted sting rays taken at Beaufort in 1909, and of another taken at an earlier date. The largest of the three (sex unknown) was taken by some fishermen in the deeper part of the outer harbor in September 1901. Its body was 2 feet 2 inches long and 4 feet wide, while the tail was 4 feet 8 inches long. The second, a female, was taken by the writer in a seine on June 12, 1909, at the Narrows of Newport River, a wide-mouthed tidal estuary whose lower reaches form part of Beaufort Harbor. When just out of the water, its measurements were as follows: length, snout to the tip of the ventrals, 18 inches; width over pectorals 26 inches; length of tail 40½ inches.

The third specimen, also a female, was, on July 3, 1909, brought to the Fisheries Laboratory by Mr. Russell J. Coles, of Danville, Virginia. A few days previously he had caught it on a hook in the Bight of Cape Lookout, and rightly thinking it to be an unusual form, had preserved it in a tank of formalin loaned him by the laboratory and had brought it in for identification. This fish was 23½ inches wide and 16 inches long, with a tail measuring 35½ inches. A fourth specimen, of the same sex as the preceding, also taken at Cape Lookout (in 1906), is recorded in the card catalogue of fishes at the Laboratory, but no measurements are given.

In 1910, I had the good fortune to get three fine specimens of this fish, at the capture of two of which I was present, while the third was brought to me some 30 minutes after death. These rays have been previously described (Gudger, 1912A), but their measurements will be given here and their markings discussed in the section dealing with color and spots.

No. 1, a female, was taken about half-way between the laboratory and the Narrows of Newport River. It was 28½ inches wide, 19 inches long, with a 33 inch tail. The total length was 49 inches and its weight 11½ pounds. It had two spines.

No. 2 was a male taken in the channel connecting the inner and outer harbors. Its width was 27½ inches, length 18½, tail 39½, total length 54½ inches. It had two spines.

No. 3, also a male, was taken within 200 yards of the hauling ground where No. 2 was seined. Its width was 37 inches; length, snout to ventrals, 26½ inches, tail only 27¾, over all 49¾ inches. The width between its eyes was 5 inches, the longest diameter of the spiracle was 1¾ inches, and its weight was over 25 pounds—the limit of my spring balance. This ray had three spines, the middle one of which was torn loose, as was the anterior spine of No. 2 above. Its tail was very short, far too short in proportion to the other dimensions if the fish be compared to a normal ray. Whether or not this abbreviation was the result of an accident, can not be said, since there was nothing to indicate the cause. Interesting points concerning the color, number, size, arrangement, and position of spots of this ray will be taken up in the section dealing with the color. This specimen was the largest and finest of the five which I have had from Beaufort waters.

Coles (1910), who (as noted above) had furnished me with a fine specimen early in July 1909, had the good fortune to take more than fifty specimens during that season. During July 1911, he saw nearly as many more but only took eight. The largest of these, a female without eggs or embryos, was 5¾ feet wide, 3 feet long, tail 5¾ feet long, total length of body 8¾ feet, weight 132 pounds.

In the summer of 1912, Mr. Coles captured a number of spotted rays at Cape Lookout. The measurements of the three largest are as follows: first specimen, a female, 9½ feet long from tip of snout to end of tail, and 7 feet 2 inches wide; the second, also a female, was 12 feet long over all, and 7 feet 7 inches wide, and 20 inches thick measured on a lance thrust through the body; the third, a male, was 10 feet long, and 6 feet 11 inches wide. It is to be regretted that measurements of the body proper and of tails alone were not made. These giant rays would probably have weighed 400 to 500 pounds each. Further consideration of these specimens will be taken up in the section on markings.

The specimens taken by Coles and myself, together with certain observations of his noted elsewhere (Coles, 1910), indicate that these rays are far more abundant in the Beaufort region than I had previously thought (Gudger 1910 and 1912A). Their relative abundance along our coasts at certain seasons of the year is also testified to by various fishermen of Beaufort and Morehead City.

My Florida specimens agree in general characters with those from North Carolina. The largest (and the largest I have ever seen) measured as follows: width 5 feet 2 inches; length to tip of ventrals 3 feet 8 inches; length of tail only 6 feet 10 inches; length all over, 9 feet 9 inches; width between eyes 8⅞ inches, between spiracles 5½ inches; weight 120 pounds. The others need not detain us here. Their measurements are given in the table on page 261. Details of color and number and size of teeth, etc., will be taken up in the sections dealing with these structures. It may be remarked in passing that these rays seem to be abundant about Key West. Dr. Mayer, in one afternoon in May, off Slaughter-house Point, secured three from fishermen using the grains. At the same place a month later I took the large one above referred to. Dr. Mayer's specimens were preserved in a rather unique way. They were small rays and this made it possible to suspend them in a can at the ice factory in Key West where they were frozen solidly in a 300-pound block of ice to await my coming some weeks later.

Figure 1, plate I, which serves as a frontispiece to this paper is reproduced from a photograph of my specimen No. 3 of 1910. Figure 2, plate I, is a ventral view of specimen No. 2 of 1910, while figure 13, plate VI, is a lateral view of Coles's 1909 specimen. My specimens were photographed while perfectly fresh, in fact within an hour after being taken. The specimen from which the lateral view was made had been in formalin for a few days, but so far as could be told was quite normal in all respects.

The photographs reproduced in figures 1 and 2, plate I, were made as follows; to a post standing just before the largest window in the laboratory a cross-piece was nailed; over this was hung a white sheet to serve as a background; in front of this the fish was suspended from the cross-piece by a cord run through the spiracles and their inter-communicating passage. The ray was first suspended by fish-hooks caught in the upper gill-slits, but as the very considerable weight of the fish caused these to tear the flesh, as shown in the ventral view, they were discarded. The method used in getting the lateral view is clear from the figure. The fin is turned up to show the gill-slits.

If figure 1, plate I, be compared with the various illustrations reproduced in this article, some interesting comparisons may be drawn. In Marcgrave's figure (text-fig. 1), it will be seen that the general outlines are good, even though the drawing is crude. His figure is drawn from a "quartering" view, *i. e.*, from a point above, but about 45 degrees to the left. He says that the whole upper part is of a steel color and that scattered over the whole of this are white spots. The anterior borders of the pectorals are too convex, the posterior edges too concave, the angles too acute. The snout is too blunt, the eyes and spiracles too high on the head. The fore and aft striations were not found in any of my specimens but are shown in Duméril's figure (fig. 8, plate IV). But with all its defects, even to a novice, text-figure 1 is plainly a drawing of an eagle ray; and when the description of the teeth is taken into account, it is unmistakably *Aëtobatus narinari.* But if comparison is made with Marcgrave's original figure, the water-color drawing shown in figure 3, plate II, the excellence of the latter will be greatly emphasized.

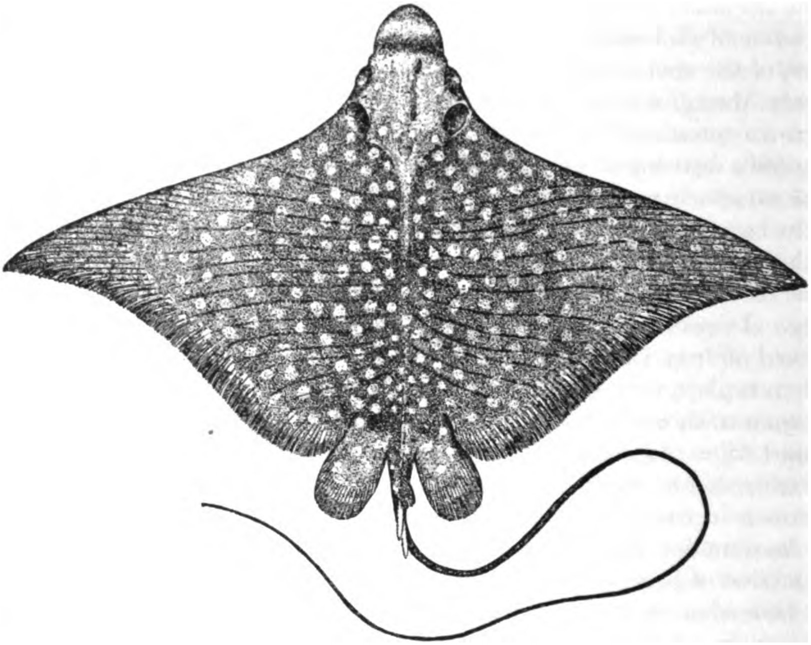
As indicated above, the older writers merely copy Marcgrave, and those who do not do worse, for quite a number of them, notably Bloch, apply the name *narinari* to rays which are not only not Aëtobatids but in some cases not even Myliobatids. It will be remembered that Euphrasen declared his figure of the spotted sting ray to be much superior to Willughby's, which is merely Marcgrave's figure redrawn and somewhat touched up. But if the figure in question (fig. 5, plate III) be compared with my photograph or Duméril's drawing of *Aëtobatus narinari* or Marcgrave's water-color drawing, some extremely grave faults will be revealed.

In figure 5, plate III, body, head, and pectorals seem to be on a level. Both the anterior and posterior edges of the wings are too flat in outline, while the ventral fins are shown as bifurcated backward prolongations of the body. Inspection of figure 1, plate I, shows them to be short and clearly marked off from the body. The dorsal fin is too long and is wrongly inserted. Figure 1, plate I, does not show it clearly, but text-figure 3 (from Jordan and Evermann) shows both size and position properly. Where Euphrasen got the fringed edges of the pectoral and ventral fins is inexplicable. It is, however, probable that his figure was drawn from a much shrunken, dried or preserved specimen in which the fin rays were very prominent; but even that would not account for their extending beyond the substance of the body proper. Inspection of figure 1, plate I, shows that the fins in question possess slightly notched edges but nothing more. Attention is also called to the exaggerated size of the spots, to their relatively small number, and to the fact that there are practically none on the head; also to the position of the spiracles and eyes, which are dorsal instead of lateral, the eyes in the fresh specimen not being visible from above. Further the snout is too short, too narrow, and too much on a level with the main part of the head.

Russell's (1803) Eel Tenkee (fig. 6, plate III) is a good drawing with some rather apparent defects from the standpoint of my specimens. The body is too flat, and the head especially so; the anterior parts lack spots, and the eyes are too prominent; posteriorly the line of demarcation between pectorals and ventrals is continued too far forward. However, the crenate posterior edges of the fins are correctly shown. This drawing was made by a native artist, but Russell especially vouches for the accuracy of the details.

Duméril's elegant figures (fig. 8, plate IV) have already been referred to on page 252. For the reasons noted elsewhere it seems to me that *A*, *Aëtobatus latirostris* is not (as Gunther asserts) *A. narinari*, but that *B* (lower left head) is *narinari.* The drawing was evidently made from a preserved specimen, in which by drying or hardening the flesh had sunk over the anterior fontanelle of the skull. The position of the spiracles is for the same reason somewhat distorted.

Gunther's excellent little outline drawing (text-fig. 2) has been previously referred to and need detain us but a moment. One's attention is forcibly called to the absence of spots on the anterior parts, to the elongated and very *rounded* ventrals, and to the exaggerated spine. The dorsal fin and head are well drawn, the eyes being almost invisible, but the spiracles are placed rather too near the mid-dorsal line.



TEXT-FIG. 3.—*A. narinari*, dorsal view, after Jordan and Evermann (1900), Florida.

The only other figures of this fish (and the only drawing of the ventral surface known prior to 1913) are those previously referred to from Jordan and Evermann's "Fishes of North and Middle America," and widely reproduced in various papers by one or the other of these ichthyologists, or by other American writers, some of whose papers will be referred to later. If text-figure 3 (their drawing) be compared with figure 1, plate 1 (my photograph), the following differences are observable: the pectorals are more sharply pointed, the ventrals more widely spread, longer, and more rounded than in my specimens. The spots are larger and fewer in number (especially on the head and the edges of the pectorals) than in Beaufort and Key West specimens. The most striking difference, however, is to be found in the absence from the latter fish of the dark, roughly parallel lines, which extend from the dorsum of the former out over the pectorals.

I have elsewhere recorded (Gudger, 1910) the difficulties I had in identifying my first specimen as *A. narinari*, but it may not be out of place to repeat them here. The lines above referred to are sometimes present in the living or at any rate in the freshly killed fish, but are so indistinct as to be found only after close search. So nearly invisible are they that my first specimen, examined while alive and in brilliant sunshine, showed no traces of them. Some hours later, when this fish was brought to the laboratory and compared with Jordan and Evermann's figure, despite its agreement with the characters given on pp. 87 and 88 of vol. 1 of their "Fishes of North and Middle America," I thought it to be a new species; and it was not until Mr. Henry D. Aller, director of the Laboratory, pointed out the very faint striations, that I was convinced to the contrary. It is significant that Jordan and Evermann make no reference whatever in their text to such lines. The photograph (fig. 1, plate 1), made from my fresh fish, shows that they are too indistinct to affect a sensitized plate, even when exposed 60 seconds. Attention may be called here to the sharpness of focus in this photograph, as even the fin rays are shown with marked clearness.

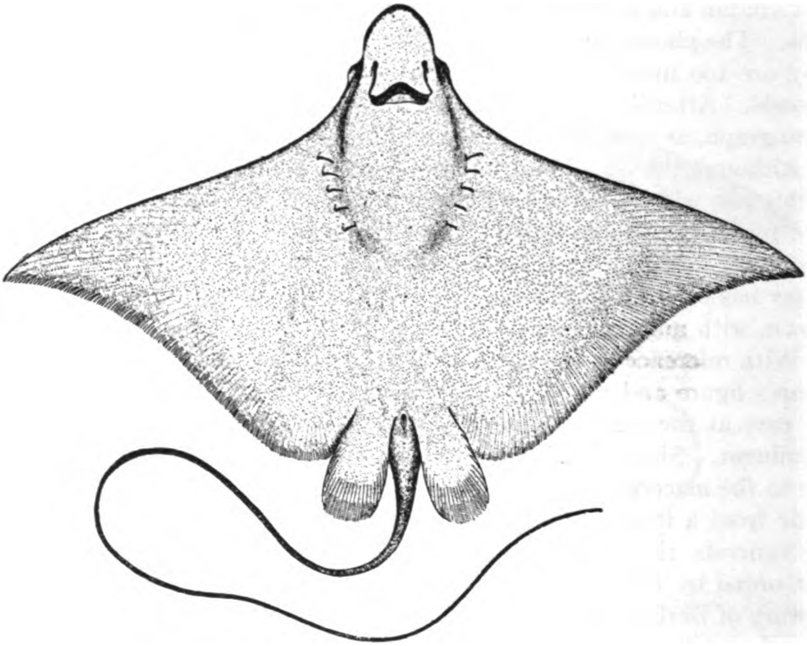
Although the question of the presence or absence of lines on the dorsum of this fish will be taken up at length later, it does not seem out of place here to refer to Abbeville's (1614) *Narinnary* with its back "all striped of black and white." Save for De Laet's (1633) quotation of Abbeville, no writer has referred to these stripes until Smith (1907) wrote: "Color above brown, with numerous small, round, pale spots, and transverse dark lines."

With reference to the fins, another difference between Jordan and Evermann's figure and my photograph should be pointed out. In the former the rays at the angles of the pectorals and on their hinder edges are very prominent. Since the drawing is from an alcoholic specimen, this must be due to the macerating action of the alcohol. In the photograph, which was made from a fresh specimen (dead an hour), such rays are visible only in the ventrals, the posterior edges of the pectorals are finely scalloped, as first noted by Russell (1803), but first shown in the painting in the Royal Library of Berlin and here reproduced as figure 3, plate II. Since particular attention will be given to the head and snout in the section bearing that title, nothing more will be done here than to call attention to the fact that the head is too light in color and too prominent. The spiracles are too marked, the eyes are too prominent and placed too high on the sides of the head, while the snout is too short and too blunt.

Figure 2, plate 1, is a photograph of the ventral surface of the fish previously described, and, with the exception of Jordan and Evermann's and Coles's (1913) figures herein reproduced, is the only one known to the writer. Leaving out of further account the various general features already spoken of, in comparing the two figures, no essential differences are noticeable save in the head.

The snout in the photograph shows to better advantage than in the dorsal view, since it is in the ventral plane of the body. That in Jordan and Evermann's figure is plainly too short and rounded. The mouth is too squarely cut, the lower lip especially so. In the photograph the nasal flaps are longer and fit more closely in the corners of the mouth. Still more marked is the sharper angle of the point of the lower jaw and its projection beyond the mouth and indeed over the lower lip. These structures were found in every fish examined by the writer.

The first and only description of our spotted sting ray from Brazilian waters since Marcgrave (1648) is that by Miranda Ribeiro (1907), 259 years later. His very clear diagnosis of the characters, both generic and specific, agrees closely with Jordan and Evermann. These Brazilian rays, however, are dark olive above with blue-white spots, which are round, more or less equidistant from each other, and in size about equal to that of the eyes.



TEXT-FIG. 4.—Ventral view of *A. narinari*, after Jordan and Evermann, 1900, Florida.

As Gunther's (1870) characterization of *A. narinari* from specimens in the British Museum is the most authoritative since Müller and Henle (1841), so his description of the single specimen in the Museum Godeffroy at Hamburg (1910) is the most satisfactory since Jordan and Evermann's (1896). His specimen from Samoa is, he says, not to be distinguished from Atlantic specimens. However, he describes its nose as blunt, whereas my figures and observations plainly show it to be sharp-pointed in all, although of varying widths towards the base. The whole upper surface of the body in his young specimen was covered with numerous bluish-white spots.

In connection with notes on the embryos of certain rays (*A. narinari* especially) taken at Cape Lookout in 1912, Coles has (1913) incidentally published some elegant figures of our ray, which, through his kindness and that of the American Museum of Natural History, are reproduced herein as figures 9, 10, and 11, plate v. One text-figure of his paper will be omitted, since it shows no points not given in figure 9, plate v. This figure is a lateral view of a female 7 feet 2 inches wide. Figures 10 and 11 are head-on and ventral views of the same fish. Figure 10 is the first head-on view ever published, and the only ventral representation except the Jordan and Evermann drawing, (text-fig. 4) is figure 11. Unfortunately this last is taken at too flat an angle to give a clear representation of the whole ventral surface. Further attention will be called to these figures in other sections.[1]

The following table gives in comparative form the measurements (in inches), absolute and relative, of the various specimens of the spotted eagle ray described by the authors previously quoted:

Author.	Length.	Breadth.	Tail.	$B = Lx$	$T = Lx$
Abbeville	72	72	72	1 ±	1 ±
Marcgrave	18	46	51	2.6	2.8
Euphrasen*	......	......	......	......	3
Bloch & Schneider	......	......	......	......	3
Ruppell	......	......	......	2	4
Russell	10	34	62	3.4	6.2
Müller & Henle	12	28	52	2.3	4.3
Forster	11	20.5	35	1.9	3.2
Cantor	7.25	11.75	33.75	1.6	4.7
Bleeker	......	......	......	2 −	3 +
Duméril	......	......	......	2	3 to 4
Klunzinger	......	......	......	1.67	3 to 3.5
Day	......	......	......	......	3 to 4
Jordan & Evermann	......	......	......	2	3 to 4
Beaufort, 1901	26	48	56	1.84	2.2
Gudger, 1909	18	26	40.5	1.4	2.3
Coles, 1909	16	23.5	35.5	1.5	2.2
Gudger (Beaufort, 1910), I	19	28.5	33	1.5	1.7
II	18.5	27.5	39.5	1.5	2.1
III	26.5	37	27.8	1.4	1
Coles, 1910	36	69	69	1.9	1.9
Jenkins	15.5	31 +	32.5	2 +	2
Miranda Ribeiro	......	......	......	2	3 to 4
Gunther	11	......	43	......	4
Coles (Cape Lookout, 1912) I	......	86	......	......	......
II	......	91	......	......	......
III	......	83	......	......	......
Gudger (Key West, 1913) I	24.5	33	37.75	1.3	1.6
II	23	32	44.5	1.4	1.9
III	24	34	60	1.4	2.5
IV	32	62	82	1.9	2.6

SPECIFIC DESCRIPTIONS.

COLOR AND MARKINGS.

Aëtobatus narinari shows such marked variations in color of both the general upper surface of the body and of the spots, that it may not be without value to summarize here the work of the various investigations previously cited and to give my rather full observations of the specimens which I have examined.

Marcgrave (1648) says that the upper part of the body is of a steel (*ferreus*) color while "scattered over the entire fish are white spots about the size of a *nummus misnicus*." This would indicate that they are found on the head, though his figure does not so show. However, they are shown on the head in the water-color painting. In his colored figure the upper surface is a dark steel-blue with white spots. Piso (1658) also speaks of its color being blue.

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[1] In an interesting book on fishing in Florida, "The Giant Fish of Florida," J. Turner-Turner (1902) describes the capture of a number of spotted sting rays. His figures, made from photographs, are quite good. His descriptions need not detain us here.

Omitting those who merely copy Marcgrave without ever having seen the fish, we next come to Sloane (1697) whose Jamaican ray was "smooth, blue, marked with white spots" on the dorsum. His slightly fuller description, dated 1725, makes the fish "all blue even in the flesh itself, with white spots on it."

Browne (1756), whose fish came from the same waters, speaks of the middle parts as bluish-mixed (*media cæruleo-miscella*).

Next comes Euphrasen (1790), whose ray (also a West Indian specimen) was a brown above with round white spots, in diameter about the size of a man's thumb. His drawing (fig. 5, plate III) has the spots too large, too few, and entirely lacking on the head. In Walbaum's "Artedi," volume 3, 1792, Euphrasen is quoted substantially as above, as he is also by Schneider (Bloch and Schneider, 1801).

Russell's (1803) ocellated ray had a dark ash-colored upper surface spotted with numerous small, round, white spots edged with black—these, however, being absent from the head.

The question whether or not Quoy and Gaimard's 5-spined ray from Guam is identical with *A. narinari* will be taken up in the section on the species, but for the sake of continuity it seems well to give their notes on the color. This was a dark brown sprinkled with round sky-blue spots.

Ruppell's (1835) Red Sea ray had its whole upper surface of dark olive variegated with white spots. He declares that his ray, of which he gives no figure, is identical with Russell's Eel Tenkee, which is shown in figure 6, plate III.

Müller and Henle (1841) examined twelve specimens from Brazil, the Red Sea, and the Indies, whose coloring was brown interspersed with white spots scattered regularly over the whole dorsal surface save the head only; but they add that the number of these spots was sometimes small.

Forster's toothless ray (Lichtenstein, 1844), which is *Aëtobatus narinari* beyond any doubt, was lead or steel colored above and "scattered over the body" were many round white spots.

The spotted sting ray found in the Malayan waters was, according to Cantor (1849), "Above greenish-olive or greenish-gray; a little behind the occiput and behind the anterior margin of the pectorals appear more or less numerous greenish-white rounded spots edged with black."

Bleeker (1852), who found these rays common throughout the East Indies, describes them as being coppery green above, the back and fins both pectoral and ventral dotted with scattering round pearly-blue spots. No definite statement is made as to their occurrence on the head.

Duméril (1861) has gone into the question of spots more carefully than any other writer. He says, describing the congeneric *A. latirostris*, which Gunther declares to be a mere variation of *A. narinari* and not entitled to specific rank (on this point see page 315 of this article): "On a brownish-black foundation are roundish white spots irregularly scattered and occupying all the upper surface of the animal." Contrasting it with *A. nari-*

nari, he notes that its spots are larger, farther apart, and hence fewer. The average diameter of 50 spots on *A. latirostris* was 8 to 9 mm., of 100 on *narinari* was 4 to 6 mm. (For other specific differences see page 315). These points are clearly brought out by figure 8, plate IV, a photographic reproduction of Duméril's drawing, inspection of which will show spots on the heads of both species.

Day's (1865) Malabar specimen had a grayish-olive back covered behind the head with numerous dirty-white spots with black edges. He unfortunately gives no figures. Duméril says of *A. narinari*, in his "Histoire Naturelle des Poissons" (1865): "General color brown, with small circular spots of whitish-green bordered by black, regularly distributed and varying in number."

In 1867, the distinguished ichthyologist, Dr. Theodore N. Gill, described an *Aëtobatus*, which had been received by the Smithsonian Institution from San Francisco, under the name *A. laticeps*. Its color was bluish black above, interspersed with numerous fairly distinct whitish or yellowish spots, smaller than the eye. These were smaller on the head and larger on the body and behind towards the sides, and were somewhat ocellated on the ventrals. The pectorals were margined with blackish, while on the best Beaufort specimens the margin is whitish. Some of the Key West specimens, however, had both margins. The specific identity of this specimen will be discussed later.

Gunther (1870), from a study of twelve specimens in the British Museum, merely says that the upper surface, whose color is not stated, is generally adorned with numerous round bluish-white spots. He decides that the variations above noted are not specific and puts all forms into one species, *narinari*. Later (1880), he continues the one species and adds that it may be easily recognized by the numerous bluish-white round spots on the dorsum, though reference to his drawing (text-fig. 2) shows that these are lacking on the head and anterior part of the pectorals.

In 1871, Klunzinger redescribed the spotted sting ray of the Red Sea. His specimen was grayish-black on the dorsal surface and everywhere (except on the head) there were round white spots, rather few in number. Ruppell's ray from the same waters, it will be remembered, was dark olive above with white spots all over the dorsum.

Day's (1878) Indian specimens (localities not noted) were grayish-olive, sometimes greenish-olive or leaden-gray, and were generally covered behind the head with dirty-white or bluish spots edged with black. To this description is added the interesting statement that in immature forms the upper parts are a deep lead color and that the spots are hardly visible.

Chronologically Jordan and Gilbert (1882) come next, but as their description is identical with that found in Jordan and Evermann (1896), which will be discussed at length later, consideration of it will be deferred.

In 1895 Henshall described a specimen from the west coast of Florida under the name *Stoasodon narinari*. It was dark brown above and thickly covered with white spots, about half an inch in diameter.

Jordan, in his "Fishes of Sinaloa" (1895), describes an *A. narinari* from the Pacific Coast of Mexico whose "Color is bluish-black with many round yellowish spots scattered equally over the back and ventral fins; spots about as large as eye on back, smaller on head; sometimes two spots run together, forming an elliptical spot [see here fig. 1, plate 1 of this paper]; about 16 spots from eye along anterior margin of pectoral to lateral angle; posterior margin of pectoral very narrowly margined with white [see my fig. 1]; ventral side pearly white."

On page 2753 of volume III (1898) of the "Fishes of North and Middle America," Jordan, over his own signature, repeats the description of his Mazatlan specimens from Sinaloa and adds that there are no noticeable differences between these (sometimes identified as *A. laticeps*) and the West Indian specimens. A careful comparison of his description with the photograph serving as a frontispiece for this paper will show how true this is for Beaufort specimens. Jordan calls particular attention to the differences between specimens from Mazatlan (above) and the original type specimen of *A. laticeps*, whose locality is unknown but which was received from San Francisco. Its color was "bluish-black with numerous rounded yellowish spots on head, smaller than eye, much larger on body, assuming on the pectoral the form of ocelli." (Page 88, vol. 1.)

Evermann and Marsh, in their "Fishes of Porto Rico" (1900), describe the life color of a specimen taken at Culebra Island, near Porto Rico: "General color of whole upper surface light chocolate brown, everywhere covered with roundish or oblong pearly or bluish spots or blotches, largest about size of eye, smallest less than half as large; under surface milky white except margin of snout, which is dark gray; tail uniform chocolate brown; iris yellowish gray." They reproduce the figures in Jordan and Evermann above referred to.

Jenkins (1904) found this beautiful ray rather common at Honolulu, but only made a critical examination of one specimen. He describes it as having a blue dorsum covered with very many clearly marked white ocellus-like spots about the size of the eye, the head in front of the spiracles being devoid of spots and therein very unlike the Beaufort specimens.

Calling our ray *Stoasodon narinari*, Jordan and Evermann (1905), in their "Fishes of the Hawaiian Islands," find specimens from Honolulu and Hilo to have exactly the same colors as those from Porto Rico. One in life was bluish-gray on the dorsal surface and slightly darker on the pectorals, while the back was covered with bluish-white spots, of which those in the middle region were largest and those on the edges of the fins smallest, exactly as may be seen in my photograph of one Beaufort specimen.

Jordan's "Guide to the Study of Fishes," volume 1 (1905), tells us that *Aëtobatus narinari* "is showily colored, brown with yellowish spots."

In collaboration with Seale (1907) Jordan describes one specimen from Cavite, Philippine Islands: "In spirits the color is brownish, the upper surface of the disk covered with pale blue spots. The pale spots are much fainter than in Hawaiian examples. The latter, however, of much larger size." Here the reader's attention is called to Duméril's statement concerning the relative size of the spots.

H. M. Smith (1907), writing of Beaufort specimens, says that the color is brown on the dorsal surface with many round, small, pale spots and with dark parallel lines [not bands] running transversely. He is the first and only writer to refer to the dark bands shown in Jordan and Evermann's drawing (text-fig. 3).

Recalling Marcgrave's (1648) statement, that his ray was iron or steel colored above, with white spots scattered all over the whole dorsal surface (except the head and snout), it is interesting to have Miranda Ribeiro's (1907) description of Brazilian specimens. Writing 259 years after Marcgrave, he says present-day specimens are, "dark olive above with blue-white spots, which are round, more or less equidistant from each other, and of a size approximately that of the eyes."

Gunther's (1910) South Sea specimen from Samoa is expressly declared to be indistinguishable from Atlantic specimens of equal size. No general color is given, but the whole upper surface is thick-set with round bluish-white spots, those on the head, however, being rather few.

The first and only writer who seems to have ever seen the lines or bars was the French Capuchin friar, Claude d'Abbeville (1614). He states briefly and simply that "this fish (the *Narinnary*) is all striped of black and white."

Excepting de Laet (1633), who merely quotes d'Abbeville, no writer from 1614 to 1907 (Smith) makes mention of these curious and interesting color bands and lines. My own observations on Beaufort and Key West specimens are given at length in the following pages, together with the conclusions based thereon.

I have had from Beaufort five specimens, two in 1909 and three in 1910. Elsewhere (Gudger, 1910 and 1912A) these various specimens have been described. In 1909 I had no knowledge of the great variations in color which are found in *Aëtobatus narinari* and unfortunately made no notes thereon, being chiefly occupied with those points wherein these specimens differed so markedly from those of Jordan and Evermann. At this late day, all that can be said is that their color agreed pretty closely with that given by these authors for their specimens. My notes, however, record the fact that in life no lines were visible on specimen No. 1. I was thoroughly convinced of their absence until Director Aller, after the fish had been dead some hours, called my attention to them. They were, however, extremely faint and only visible when the light fell on the fish at a certain angle. The specimen was dissected for internal organs and jaws and thrown away.

Mr. Coles's specimen, No. 2 for 1909, after being in 5 per cent formalin for a year, was carefully examined. Its general color was found to be a light brown overlaid with a bluish or leaden-gray. The spots which were scattered over the whole dorsal surface (head included) were cream-colored or dirty white. None of them had dark rings around them, but nearly all had dim white centers, giving the ocelli-like appearance. These spots were larger on the back and smaller on the head and outer parts of the pectorals, while near the ventral fins a few ran together to make elliptical or dumb-bell-shaped spots. The right pectoral had on its anterior edge 23 spots, on its posterior 25; the left pectoral had in front 20 and behind 27 spots, not all of which were of approximately equal size. The dorsal had on each side a large spot with a dark hinder edge, and at its base another large spot on each side. On either side of the tail, just under the spines, was a white stripe. The bands shown in Jordan and Evermann's drawing (text-fig. 3), which were not visible to the eye when the specimen was brought to the laboratory and which are not to be found in a photograph of this ray made with a very small diaphragm and a long exposure, were at this time visible as very faint lines, having no width, not bands. This was a male, probably immature, having claspers only half an inch long, pointed at the ends, and with very faint grooves.

Specimen No. I, 1910, was taken June 30 in Newport River, about 4 miles from the laboratory. Its measurements, as well as those of Nos. II and III (to be described later), are given in the table on page 261. In bringing it into the laboratory, it was badly handled and sunburned, resulting in a loss of cuticle in places, but the color could be made out fairly satisfactorily.

In the fresh specimen the ground color was a dark chestnut or brown and the spots a rich yellow cream. These spots were found over all the body, but were smaller on the head, while some few in the pectoral region had run together. The fish was put in a boat and partly covered with water. The cream-colored spots gradually became whitish. That part of the fish out of the water turned dark and finally became a velvety black and the spots *blue*. The dark cuticle peeled off during the day, leaving the ground color a dirty brown and the spots white. In the fresh (just dead) specimens no bands could be found, but after death faint lines could be made out by close inspection; these, however, did not cross the spots as shown by Jordan and Evermann (text-fig. 3), but were rather outlined and marked off by them.

After being in 5 per cent formalin for 19 days, this fish showed the following colorations: The body generally was of a light chocolate brown with bluish-gray regions where the sunburn was not bad (the cuticle gave the grayish or lead-blue color); the spots were white varying to creamy white, some with bright white centers, giving the ocellus-like appearance previously reported. As noted in the first specimen, the spots on the head were smaller than these in the mid-pectoral region—the mid-dorsal and outer pectoral regions were too badly mauled to have their spots described. The spots extended in on the ventral surface of the spiracles. On the right pectoral, anterior edge, there were 17 + spots, and on the same surface of the left fin 18 + spots. Since the tips of the fins were badly split, the numbers must be marked +. The dorsal was devoid of spots on and below it. Below the spines there was a white line or stripe on either side. The transverse lines previously referred to were present and pretty definite. They were extremely narrow bands made by dirty white lines with dark edges, due to the ground color. On none of my specimens were they so marked as on this one.

Specimen No. II was taken in the channel connecting the inner and outer harbors, on July 4. When it came in I was very busy with other matters, so it was put in a tank of running salt water and left for two or three hours. When it was got round to, I found to my regret that the cuticle was beginning to slip, so all haste was made to photograph it. This took so much time that no careful notes of the life color could be made. The ventral view (fig. 2, plate I), the front aspect (fig. 16, plate VI), and the lateral view of the head (fig. 12, plate VI) were all made from this fish.

After immersion in formalin (5 per cent) for 15 days, the color of this specimen showed up as follows: general color pearl gray or bluish-gray; where the cuticle had slipped it was a light chocolate brown; the spots, larger on mid-dorsal region and smaller on the head and at the extremities of the pectorals, were a bright white in the center of the disk and elsewhere a dirty white with distinct white centers, giving the familiar eye-like appearance. Sparsely scattered over the whole disk were dumb-bell-shaped markings made by the coalescence of two spots. The dorsal fin had on its hinder half the familiar white spot changed to a stripe, edged with black, and at its base a large spot. Both pectorals had spots as follows: Anterior edge, 20 spots; posterior, 23; and in both a line of very indistinct spots on the crenate hinder edges. The outer and inner edges of the bases of the ventrals were edged with white. White spots were found in the spiracles as in other specimens. The transverse lines were fairly distinct and in some cases they crossed the spots. This was an immature male, with claspers only three-fourths of an inch long.

Spotted sting ray No. III, 1910, was taken, on July 7, within 300 yards of the locality for No. II, by the same fishermen. I reached the scene while it was still flapping on the beach, and, as soon as it was dead, covered it with wet towels and bringing it to the laboratory (a third of a mile away) photographed it while still fresh. Its picture forms the frontispiece to this paper. While fresh its color was a dark chocolate-brown and its spots were cream-colored, some of them turning a faint bluish or greenish-blue. No striations whatever could be found although they were carefully searched for. After 12 days in weak formalin its condition was almost as perfect as when brought in, but its color was now a bluish-gray while the spots were still cream-colored running to white. This ray had the largest spots of any examined at Beaufort by the writer. Smaller on the head where they were arranged in definite rows, they were pretty uniform in size over the rest of the body, and (more than on any other Beaufort fish examined by me) showed a tendency to run together (see fig. 1, plate I).

Attention is called to the spots of extra large size at the base of the ventrals and to their presence in the spiracles. On the anterior edge of the right pectoral fin there were 20 spots, and in the same region on the left 21. It was not easy to count them on the posterior edges, because especially on the right fin they were so mixed up with the line of smaller and fainter ones formed on the extreme edges in the crenations, and further because the inturnings of these fins next to the ventrals had the spots confluent to form white stripes or splotches. The regular arrangement of these spots in very definite rows was noticeable. These spots, which were of very uniform size throughout, were surrounded by faint, dark circles, thus giving the ocellus-like appearance recorded by so many authors. None of them had the whiter centers noted in my other specimens.

The dorsal fin had two large spots at its base and on its hinder surface a white splotch edged with black. Under the spines was a white streak, and below that (in the middle of the side of the tail) a long, pointed, dark streak. Careful examination of this specimen after immersion in formalin showed the presence of faint transverse striations on the mid-pectoral region. However, the only lines visible in the photograph (fig. 1, plate I) are fin rays.

Examination of the photograph of one of Mr. Coles's huge specimens taken in 1912 shows that this ray had on the dorsal fin a large white splotch and back of this a black border. At the base of the fin is another large white splotch, beneath this a long dark streak ending in a backwardly directed point, and lower still the white side of the tail (see fig. 9, plate V).

Attention will now be called to the spots on the dorsal surfaces of these rays. One of these, a male 6 feet 11 inches wide, had, in the region of the bases of the pelvic fins and at the root of the tail, a few irregularly shaped markings formed by the coalesence of two or more spots, but no distinct ocellations. Another, a female 7 feet 7 inches wide, had practically the whole dorsal surface covered with white spots with dark centers. However, it is a third specimen, a female 7 feet 2 inches wide, in which these ocellated spots are most perfectly developed. Figures 9, 10, and 11, plate V, are lateral, frontal, and ventral views of this ray. Study of these figures shows that these eyed spots are formed by two or more spots coalescing to form U or C-shaped spots and that these finally close up to make white rings with dark centers.

There now remain to be described the four Florida specimens. Three of these, it will be remembered, were taken at Key West by Dr. Mayer on May 10 and were preserved by freezing in a large block of ice. On May 29 the first of these, a male 2¾ feet wide, was examined. The epidermis on this specimen was beginning to slip, but those parts still covered by it were a blackish-brown, those from which it had slipped were a lavender gray. The spots were found all over the dorsum, but those on the head, and especially in front of the eyes, were smaller than those on the other parts of the body. In the middle parts of the back and on the wings the spots were larger and many were dumb-bell-shaped. An average spot measured 11 by 13 mm. There were about 27 spots along the front edge of the right pectoral. When the epidermis was pulled off the spots in it were a smoky-gray, those in the underlying skin were cream-colored. No transverse lines were visible either in the epidermis or in the true skin.

Specimen No. 11, for 1913, was also a male, 2⅚ feet wide, and was examined on the same day as the preceding. With the epidermis on its color was a blackish-brown; with this off it was a leaden-gray. The spots were cream-colored with dark centers. In the pulled-off epidermis they were whitish with dark centers, and those in the flesh were of about the same color, but were surrounded with fairly distinct dark-gray circles well marked off from the general lead-gray body color. The spots over the central part of the body ranged in shape and size from round ones having an average diameter of 10 mm. to elliptical ones 10 by 18 mm. Those on the head, between the eyes and extending into the spiracles, were smaller. Those along the front edge of the left pectoral were about 20 in number. No transverse bars were visible.

The third of the rays preserved in ice was not examined until June 7. It was a male and measured 2 feet 10 inches between the tips of its pectorals. Nearly all of the epidermis was gone from this specimen, but those parts still covered by it were blackish, while the others were a lead or steel-gray. In the epidermis the spots were light gray and on the skin whitish with dark centers. Each of the latter spots was surrounded with a dark ring about half as wide as the spot. On the central and hinder parts of the body the spots ran together to form oblong or dumb-bell-shaped spots. On the front edge of the right pectoral about 27 spots could be counted.

Transverse lines were visible—lines not bands. On running my finger along them I found that they were formed by transverse canals in the flesh just under the skin, which could be made to swell with the contained liquid. For the most part these were about an inch apart and traversed both spots and interspaces. On the hinder part of the back they ran into the large longitudinal canals marking off the outside limits of the abdomen.

On the base of the tail a gray streak on each side ran back to the spines. The dorsal was gray in front, black behind, and had a white spot at the base. The tail was white on the sides and underneath back to the end of the last spine; thence black to the tip. However, about a foot behind the dorsal there was a white spot on the right and two on the left side. This I had not noticed on any specimens before.

My fourth Florida specimen was grained in the same locality as the preceding, *i. e.*, off Slaughter-house Point, Key West Harbor, on June 20. I carried it at once to a wharf and noted its color before it was fully dead. It was a full-grown male, 5 feet 2 inches wide. Its life color was a light chocolate brown, darker toward the hinder edges of the fins, and the spots were creamy white with dark centers. On the center of the back and on the hinder parts of the body the spots were in the shape of dumb-bells, figures 3 or 8, letters S, C, and U, and in oblong and roundish markings. All the round spots had dark centers, but no outer circles were noted. However, small round spots were found everywhere among the markings just described. The large round spots are formed, as the fish grows older, by the coalescence of the horns of the U-shaped markings. All sorts of gradations of these could be found. The hinder edges of the fins were fimbriated and were margined with black, having in front of the black edge an ornamentation of spots, figures, and wavy lines.

In the cephalic region the spots were smaller, but covered the whole head, snout, and cephalic fins, and extended into the spiracles, covering both walls and flaps. The dorsal fin was white edged with black, having in front and along each side of the base a broad dark mass, and having a dark bar on each side extending from above in front obliquely backward and downward, dividing the white area into two regions, each of which had a small, round, black spot in it. The under side of the tail was white like the body, and on the sides, about a foot behind the last spine, were a number of white spots as noted on the preceding fish.

The above are all life colors. After exposure to the sun for some time, the skin turned black and the spots became greenish and finally a faint pale blue. No lines nor bands were at any time visible. For a figure of a spotted sting ray showing markings similar to those above noted see figure 9, plate v, from Coles's 1913 paper.

The foregoing diverse facts with regard to the color and markings of *Aëtobatus narinari* may enable us to understand the great differences in color which have been recorded by the authors cited. It would seem that there are four things to be considered in reconciling these differences: (*a*) that there are great variations in the color of the fish; (*b*) whether the fish was alive or dead, and if dead how long; (*c*) whether the fish was fresh or preserved, and if preserved in what and how long; (*d*) the age of the fish as shown by its relative size.

There can be no difference of opinion about the fact of great natural variations in ground color and markings. My own observations on Beaufort fish, made on five specimens (three of them alive) and on Coles's photographs, and on four specimens from Florida, show that considerable diversity exists in our form. That this is true for those in other waters is shown by the quotations previously given from various authors. Further, there can be no doubt that after death the color changes. My living fish were for the most part brown in color, but after death showed a tendency to change color, becoming dark or bluish. Preserved specimens in formalin all turned bluish-gray or lead-colored, this being due to changes in the cuticle, for when this was stripped off the general color was brown. Not enough data have been collected to show what effect age has on the color of the ray, but it is generally the rule that the older fish may be expected to be darker in color.

From the observations made by Mr. Coles and myself, it seems to be the rule that in young fish the markings are regular, but that as the fish grow older the spots become confluent to form various bizarre figures. These are found mainly in the hinder part of the body, where possibly growth is more rapid. Such irregular figures are noticeable on Coles's 1912 specimens (see fig. 9, plate v) and on my largest Florida ray.

Since Annandale's later notes on *A. narinari* (1910) go far toward clearing up the vexed question of color, I can not do better than quote them *in extenso:*

> In Edinburgh and London there are Indian specimens that agree closely with American and South Sea specimens in the British Museum, while an old female from the Madras coast differs in more respects than one from all other specimens I have seen. It appears, however, that if very old and very young individuals, in both of which the spots are obscure or absent, are omitted from consideration, three colour varieties may be distinguished, as follows:
>
> Var. *A.*—Entire dorsal surface of disk including snout, spotted.
>
> Var. *B.*—Spots on the dorsal surface confined to the post-spiracular part of the disk.
>
> Var. *C.*—Spots confluent into short transverse streaks.
>
> Var. *B* is the common variety in the northern parts of the Bay of Bengal, but is by no means confined to Indian seas. Var. *A* is found off the Coromandel and Malabar coasts as well as in the Atlantic and South Pacific; while Var. *C* is probably liable to occur in diverse places as an individual sport.
>
> The large female recently taken by the *Golden Crown* is practically devoid of spots, which appear to have become almost obsolete. Very young individuals are also unspotted; but in them the spots are just beginning to appear.

In this connection it should be noted that every specimen taken thus far by Coles or myself belongs to Annandale's variety *A*, being spotted all over the dorsal surface, *snout included.* So far as I know no Atlantic or Gulf Coast specimen has ever been taken which would belong to variety *B*. Variety *C* at first blush would seem to offer an explanation for the stripes or bands sometimes found on our specimens, but it seems to have streaks only and not spots. In this connection mention should be made of *Myliobatis asperrimus*, a beautiful ray from the Bay of Panama, which has its dorsal surface marked off by 8 to 10 transverse bluish-white bars and by numerous round, white spots. This ray was first described by Jordan and Evermann (1898), but they quoted the then unpublished paper of Gilbert and Starks, which appeared in 1904 under the title "The Fishes of Panama Bay" and which contains an elegant figure of the ray.

I have been able to find but four records for young specimens of *Aëtobatus narinari.* Jordan (1895), in his "Fishes of Sinaloa," says that he has had five "large" specimens, each 15 inches long exclusive of the tail. Seven Beaufort rays run in length 16, 18, 18½, 19, 26, 26½, and 36 inches; while four from Key West measured 23, 24, 24½, and 44 inches. Of these only the last of each set can be called large. Neither Jordan's specimens nor the smaller ones noted above can be so designated, and it is quite certain that all were very young. The males of all my specimens, save the big one from Key West, had very short claspers, indicating immaturity. This one was 5 feet 2 inches wide and had claspers 3¾ inches long. These, however, are considerably shorter than such appendages on a *Dasyatis* ray of the same size.

As corroboratory evidence of the youth of these rays the following facts may be cited. Elsewhere (Gudger 1910) the writer has reported the bringing into the world by Cæsarian operation of two young *Rhinoptera* *bonasus*, 8½ inches long by 13½ wide. Bleeker has in the same way obtained the young of *R. javanica* 20 inches wide. Later (Gudger 1912A) the writer captured by seining young *R. bonasus* only 13 inches long. There is but little difference in size between the adults of these two rays, this, if any, probably being in favor of *Aëtobatus*. If it is agreed that the fish noted in the preceding paragraph are young, then attention is called to the fact that all were normal in coloring, mine all having spots over the *whole* dorsal surface.

Günther (1910) corroborates this by his description of a young male from the South Seas, which measured 11 inches (long presumably) and whose tail was 43 inches long. Its whole upper surface was beset with round spots, a few being found on the head. However, Cantor (1849) had a young specimen from Indian waters which was only 7¼ inches long by 11¾ wide with a 2-spined tail 33¾ inches in length. This was greenish-olive or greenish-gray above and lacked spots on the head and anterior margin of the pectorals. Likewise, Day (1878) writes that in the young of the Indian form, in which the greenish-olive or leaden-gray adults have the body usually covered with numerous dirty-white or bluish spots, the back is of a deep leaden color and the spots are hardly apparent.

Annandale (1910) tells us that the very young specimens of *A. narinari* are either unspotted or have spots just beginning to appear, thus corroborating Cantor and Day. He had two Indian specimens whose respective measurements were: breadth 23.2 and 20.4 cm.; length 13.7 and 12.4 cm.; and whose tails measured 57 and 47.5 cm. long. His fine figure of the larger of these young males is reproduced herein as figure 17, plate VII. It is entirely devoid of spots, whereas both Jordan's smallest specimens and mine also were beautifully marked.

Klunzinger (1871) is the only student of this ray who has obtained an unborn young, and he contents himself with saying that it measured 12 centimeters. At Cape Lookout, in 1912, Coles (1913) was so very fortunate as to witness the giving birth to several young by a female which he had just caught. These young were uniformly spotted all over the body, the spots being fewer in the head region; these may be seen in figures 9 and 11, plate V, while figure 18, plate VII, is a photograph of one of these young taken after some months' preservation in formalin or alcohol. It will be noticed that the spots are much fainter and seemingly fewer in the preserved specimen. This particular little ray was 286 mm. wide, 171 mm. long, while its tail measured 634 mm.

The question of the presence or absence of lines or bands across the dorsal surface of the spotted eagle ray is a most interesting and puzzling one. They are sometimes present, usually absent; they are sometimes bands, generally lines when present at all. In connection with the description of one of the specimens from Florida, an explanation of the lines on its dorsum has been given (page 269). They are due to the presence between the skin and the flesh of canals filled with liquid, possibly blood.

However, some fish have bands, and on the last day of my stay at Beaufort in 1910 I believe I hit upon the proper explanation. In pulling off some of the skin from the back of a formalin specimen, I found that under the bands in the skin there were distinctly marked-off brown stripes in the flesh. It may be that these two explanations are based on two different manifestations of the same phenomenon, namely that these underlying vessels not only mark off and give rise to the transverse lines, but that after death or in preserved specimens the coloring matter from their liquid contents (perhaps the hemoglobin of blood) soaks out, discoloring the overlying flesh and skin, thus causing the formation of bands.

The facts are that in life these lines, stripes, or bands are either wanting or so faint as to be invisible not only to the eye but also to the photographic plate after a long exposure with good light and with a very small diaphragm; whereas after death, and especially after preservation in formalin, these markings are apt to show up fairly clearly. What effect immersion in alcohol would have, can only be conjectured, but it is quite certain that the specimen from which Jordan and Evermann's figure was drawn had for a considerable time been in alcohol. However, Claude d'Abbeville (1614) says that the whole (upper?) body is covered with black and white lines, and his specimen or specimens would hardly have been other than fresh. But on the other hand Mr. Coles says positively that after examining more than 100 fresh specimens at Cape Lookout he has never yet seen one with transverse bars or bands.

Before leaving the question of markings, it should be noted that it has been reported by various authors that the hinder edges of the pectorals in *A. narinari* are margined with white, while by others they are reported to be black. Indeed, the present writer has himself noted both kinds of margins. The explanation for this apparent anomaly seems to be as follows: The fish grows to some extent by additions to the hinder edges of the pectorals. On these edges the spots form fairly regular rows. Consequently at one stage of development of the fish, the pectorals will be margined with white; a little later, as the fish grows, these white margins form spots which apparently move forward and the margin is found to be black or at any rate darkish. In various specimens all such gradations may be found.

During the summer of 1913, Mr. Coles took at Cape Lookout two abnormally colored specimens of *A. narinari*, the data concerning which he has kindly put at my disposal. The first was an albino female 3 feet 4 inches wide, 2 feet 6½ inches long snout to ventrals, and 5 feet 10½ inches over all. Swimming in water 3 feet deep, this fish appeared to be perfectly white, but after capture (in a seine) it was seen to be covered with light olive and brownish-green markings, which became plainer as the body was exposed to the air and sun. Description can not do justice to this beautiful specimen, which is shown in plate VI, figure 12*A*.

On the following day, Mr. Coles took, almost in the same place, another *A. narinari*, which at first seemed to be totally black, but exposure to the light and air revealed the presence of a few small, widely scattered light spots. Since showing me the photograph, Mr. Coles has misplaced both it and the film, and it can not be reproduced here. This fish was a male 4 feet 9 inches wide, 3 feet 2 inches long, and 8 feet 5 inches long over all.

From all the data cited in this section it is clear that the spotted eagle ray is subject to wide variation in color and markings.

TAIL AND SPINES.

On a preceding page there is a table in which the lengths of the tails of the various specimens previously described are given both in absolute measurement and in proportion to the length of the body. Very great variations and discrepancies are met with, and none greater than in the account of the first describer. Abbeville (1614) writes that his fish is 6 feet (*pieds*) wide and as many long and has a tail a fathom (*brasse*) long with a spine about a full foot (*un grand pied*) long, whereupon he unnecessarily adds "and very dangerous."

Marcgrave's specimen had a tail 5 inches thick at the root and 4¼ feet long, armed with two spines 3 inches long, curved like fishhooks, and placed just behind the inch-long dorsal. His figure and the water-color painting from which it was reproduced both have the spines with a single barb on each pointing toward the head.

Piso (1648) says that the spine approaches the form of an arrowhead.

Omitting reference to those older authors, who quote Marcgrave without adding anything to our knowledge, we next take up Sloane's (1725) illuminating description of the tail of the whip ray:

> The tail was six Foot long, black, small and smooth, of which are made Whips, whence the name Whip-Ray, beyond the Pinna at the End of the Body or in the Beginning of the tail lie one, two, or three Inch and half long flat streight Bones or Radij, they are white, serrated with Teeth on both Sides like a Saw, made so as an Arrow that's bearded, to enter the Flesh easily but not to come out without tearing it, they lie one on another on the upper Part of the Tail, where there is a Hollow or Cavity made to receive them like a Sheath, that they may swim with less Impediment, and only use them on Occasion.

If the reader will now turn to figure 7, plate IV, wherein are shown Quoy and Gaimard's five-stinged tail and my Beaufort specimen with four stings, he will see how exactly Sloane has described tail and stings. However, Sloane is in error in placing the spines in a hollow or cavity on the upper part of the tail. In the large dried tail, referred to above and described later, there is no distinct cavity, but there is a flattening with a very slight concave surface due probably to drying. The spines, of course, offer no impediment to the fish's movements.

Save Sloane only, none of the older writers give so good a description of the tail as Euphrasen (1790). His preserved specimen had a tail flat, whip-like, growing smaller from base to tip, three times longer than the body, bearing at its base a small subtriangular dorsal fin. Behind this were two stings, the hinder twice as long as the anterior, both flattened sidewise with their barbs turned backward. His figure (fig. 5, plate III) is not so good as his description; the stings are entirely too long, the shafts too slender, and the barbs too fine and long.

Russell's Eel Tenkee (1803) had a "tail of great length" (5 feet 2 inches long compared to a body width of 2 feet 10 inches), tapering to a fine point, darker in color than the body (true of all the specimens I have examined), bearing a small dorsal fin and behind it a spine. In his drawing, both fin and spine are placed too far forward, between the ventral fins.

Quoy and Gaimard's (1824) 5-spined tail, previously referred to, is reproduced as *A* in figure 7, plate IV; *B* in the same figure is a dried tail presented to me by Mr. W. H. Shelton, of Beaufort. The photograph was made by laying the tail on the plate in the Atlas of the "Voyage of the *Uranie*," and having put a piece of black paper underneath the white spines, a long exposure was made with a very small diaphragm. Of their specimen, which has the largest number of spines on record of any sting ray of any kind, these authors state that "the particular form of its tail, armed with five very long spines barbed and hooked along the edges, leads us to name it the ray of the five spines, *Raia quinqueaculata*." In the section on species, the question whether or not this is an *Aëtobatus* will be taken up. Suffice it to say here that it is undoubtedly a whip-tailed ray and possibly an *Aëtobatus*.

Ruppell's (1835) *Myliobatis eeltenkee*, from the Red Sea, is identical with Russell's Indian ray from which the specific name is taken. Beyond noting that the spine is "robust" and the tail four times the length of the body, there is nothing in his description to detain us.

Müller and Henle's (1841) description of *Aëtobatis narinari* is wonderfully accurate in all points. Of the tail, which is more than three times the length of the body, they say that in the region anterior to the dorsal fin it is triangular in section, while behind the spine it is compressed and has distinct lateral grooves. These structures, with the exception of the lateral grooves, I find present in the 4-spined tail previously referred to; while the triangular form of the base of the tail extends to the end of the last and longest spine. The lateral grooves are found, however, in another dried tail in my possession.

Lichtenstein (1844) tells us that Forster's *Raja edentula* had a very long, attenuate, cylindrical tail, three times the length of the body of the fish; while behind the short dorsal were twin spines hooked and biserrate to the very tip.

When Gunther (1870) made his "Catalogue of the Fishes in the British Museum," he found therein two (dried?) tails, but, beyond noting that one had four and the other five spines, he gives no description of them.

Klunzinger (1871) describes the tail of the spotted sting ray from the Red Sea as thick only at the root, long, whiplash-like, slightly compressed, and smooth. The pointed, saw-toothed spine, which is twice the length of the eye, is situated on the base of the tail just anterior to the hinder edge of the ventral fins. Day (1878) is the only author who notes with Müller and Henle that the base of the tail is triangular as far as the spine.

Jordan and Evermann (1896) say of the eagle rays in general that they have long whiplash tails with a spine behind the dorsal fin, while nothing is said of the spines of *Aëtobatus narinari*. Later, however, Jordan himself, in volume III (1898), says of *A. laticeps* (Gill), which he believes to be identical with *A. narinari*, that its anterior caudal spine equals the length of the base of the dorsal, which in turn is half the length of the second spine. This the present writer has found to be true of every 2-spined tail examined. Euphrasen, however, was the first to note this peculiarity. Miranda Ribeiro (1907) says that the filiform tail may have from one to five spines behind the small dorsal, but Gunther (1910) affirms that one is the rule though there may be more.

The tail of *Aëtobatus narinari* is always armed with one and ordinarily with two or three spines, while the number may rise to four or five as recorded by Gunther (1870) and as shown in figure 7, plate IV, of my best Bieaufort specimen and of Quoy and Gaimard's plate, which has been previously described. My notes on the tail structures of Beaufort specimens are not so full as could be wished for, but the following data are given to supplement what has preceded. My first specimen, taken June 12, 1909, had a tail 40½ inches long, armed with two spines. My second, taken by Coles at Cape Lookout July 3 of the same year, had a tail 35½ inches in length, likewise bearing two spines, the anterior three-fourths of an inch long, the posterior 2⅛ inches.

During 1910, I examined three fine specimens. The first had a 33-inch tail bearing two spines, the first of which measured 1 inch, the second 2⅛. Specimen No. 2 possessed a tail 39½ inches long, bearing only one spine and it but three-fourths of an inch long. This was evidently a regenerating structure, for the faint groove back of the dorsal showed plainly that a spine or spines had been torn out. My best Beaufort specimen for 1910 was No. 3, which came into my hands while yet alive. Its tail bore three spines, the anterior 1¼ inches long, the middle one (nearly torn off in the net) 1¼ inches, the posterior 2 inches in length. This torn-off sting had two roots.

Mr. Coles's (1910) largest spotted ray was 5¾ feet wide, 3 feet in length, and had a tail 5¾ feet long bearing four spines, but these were unfortunately not measured. Dr. R. E. Coker, when director of the Beaufort Laboratory, recorded in 1901 the capture in the outer harbor of a spotted ray 4 feet wide and 2 feet 2 inches long, with a tail 4 feet 8 inches in length. Tail and spines (which have been excised) are preserved in the museum of the station. The short sting is 2¾ inches long and has two short roots. The longer sting measured 4⅞ inches and has a blunted single root which bears evidence of having been cut off with a knife. The tail is black and has a slight ventral keel.

The 4-spined tail, previously referred to and shown in figure 7, plate IV, is, after 2½ years of drying, 4 feet long. Of its four spines the first is 2 inches long, the second (which lacks a fraction of the tip) is 4⅛ inches, the third 5 inches, and the fourth is 6⅛ inches. The base of the tail as far backward as the tip of the second spine is triangular in cross-section. Behind that it is rectangular with the top and bottom planes slightly bowed or arched outward. The tails of Mr. Coles's huge specimens of 1912 (see page 268) each bore four spines. These are shown for one specimen in figure 9, plate v.

The data given above for the writer's own specimens are from Beaufort rays. Now there will be given similar data for Key West fish taken in 1913. Ray No. I was 2¾ feet wide, 2 feet ½ inch long, and had a tail measuring 3 feet 1¾ inches. Its anterior spine was 1½, its posterior 1¾ inches long.

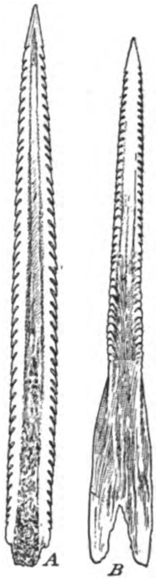
Ray No. II, 2⅝ feet wide, 1 foot 11 inches long, had a tail 3 feet 8½ inches in length. Its three spines were respectively 1⅝, 2, and 1⅝ inches long. No. III was 2 feet 10 inches in width, 2 feet in length, with a tail measuring 5 feet. Its three spines going from front to back were 1⅞ inches, 2¼, and 1⅞ inches.

Key West ray No. IV was much the finest specimen I have ever had, approximating in size Coles's giant specimens from Cape Lookout. Its width from tip to tip of pectorals was 5 feet 2 inches, length from tip of snout to end of ventrals 3⅝ feet, length of tail only 6 feet 10 inches, length all over 9¾ feet, weight 120 pounds. Like my best Beaufort specimen, it had four spines, but the front spine, torn loose and hanging by a shred of skin, measured only 2 inches; the others were perfectly normal and measured 4⅜, 5, and 4⅜ inches long.

Various authors, notably Jordan and Evermann (1898), give as a specific character of *Aëtobatus narinari* that the second spine is twice the length of the first. Where there are more than two, and especially when there are as many as four or five spines, there is a fairly regular gradation in size. Coles (1910) says that if one spine is torn out the one immediately in front grows larger to take its place. My own observations are confirmatory of this point.

It should be noted here, however, that, perhaps as the result of this multiple spine formation, the largest spine of the spotted ray is uniformly smaller than that in a Stingaree of the same size. The ordinary sting rays, however, not infrequently have two spines, in which case generally, if not always, the anterior sting is the larger. This is true of the several dried tails in the possession of the writer, one of which has four stings.

Attention is called just here to an interesting difference in the root structure of the spines of these two kinds of rays, which structure has never, so far as the writer knows, been recorded. Text-figure 5 is a photograph of two of these spines: Fig. *A* represents that of *Dasyatis say*, Fig. *B* that of *Aëtobatus narinari*. The sting of *Dasyatis say* which is torn off with diffi-



TEXT-FIG. 5.

culty, seems to have no definite bony root, but to be connected with the tough, leathery skin of the tail by a single growth. The spine of *A. narinari*, which is easily torn from the tail, has a bifurcated root in all the Beaufort and Key West specimens examined by me. In confirmation of this point see page 276 for description of the spines of this ray preserved by Dr. Coker in 1901. One has a double root, while the other has had the lower end cut off. The only other figure of such a spine is that of *Myliobatis punctatus*, figured but not described by Maclay and Macleay (1886). This is reproduced here in figure 21, plate VIII. It is bifurcated also, but less markedly so than that of *A. narinari*, *B* in text-figure 5.

The single dorsal fin is situated on the root of the tail just before the spines. The tail under it is triangular in cross-section, the dorsal being situated on the base of the triangle. In the photograph of the 4-spined tail from Beaufort the anterior part of the fin has been cut off, but the posterior portion shows a marking of which I have nowhere found mention and which, being found in every specimen save one (and it in a bad state of preservation) which I have critically examined, seems to be quite constant. This is the white splotch placed more or less vertically on the hinder edge of the dorsal, but having around it always a dark margin.[1] In small specimens this splotch covers the greater portion of the fin, but in old and large fish it is mainly in the upper and hinder portion. Other markings, which I have noted on the tails of all these rays examined carefully, are a white spot (occasionally two) at the base of the dorsal fin, a long white splotch or streak underneath the spines, and below this (on the side of the tail) a long, pointed, dark streak. This is true of Key West rays and of Mr. Coles's huge specimens taken in 1912 (see fig. 9, plate V). Klunzinger (1871) notes that his Red Sea specimen had a tail which was black everywhere save underneath the root, where it was white.

Another curious marking which I have found on tails of both small and large specimens from Beaufort and Key West, results from the presence of irregular, discontinuous dark indentations running vertically on the sides of the tail from mid-dorsal to mid-ventral line, thus giving the tail an appearance which may perhaps be best described as segmented. These indentations are, in some cases at least, opposite each other near the base of the tail, but farther away are placed "staggered," *i. e.*, one on the right placed about half-way between two on the left, and at a considerable distance from each other. At the base of the tail, near the spines, these indentations may be as near as half an inch, but toward the tip the spaces inclosed may be as much as 2 or 3 inches long. On all the Key West specimens these markings are very clear. I find these markings also on the dried tails of another Beaufort Myliobatid, *Rhinoptera bonasus*.

But one record of this has come to light. Jordan and Evermann (1898) in volume III of their "Fishes of North and Middle America," speak of the tail of a Panama ray, *Myliobatis asperrimus*, heretofore referred to, as "crossed by numerous narrow grooves, or indented lines, mostly convex forward, somewhat irregular in position and direction, and not corresponding on the two sides. In the type they follow at an average interval of about 10 mm." See also Gilbert and Starks (1904). This marking may be a family character. It certainly is a curious phenomenon and worthy of further investigation.

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[1] This spot is figured but not referred to in Duméril's *A. latirostris*, fig. 8, plate IV.

The tail of the spotted eagle ray is long, slender, whip-like, and behind the region of the spines is dark in color and often a velvety black. In life it is rounded or but slightly flattened, but dried or preserved specimens, which have been hardened or shrunk by the preservative, are, as previously noted, triangular in cross-section in the region of the dorsal fin and spines, while further back they are rather rectangular in shape with the dorsi-ventral axis about twice as long as the horizontal one. The dorsal and ventral surfaces in dried specimens are slightly curved outward, while the sides are often insunken. At the hinder part of the dorsal flattening, immediately under the spines, there is a (slight) cavity as first noted by Sloane (1725) and by no one else. However, in none of the specimens examined by the writer is it large enough to receive even the one spine immediately over it, much less the whole collection, as Sloane thought. His idea that the ray thus concealed the spines lest they should be an impediment while swimming has of course no foundation. See his statement on page 274.

The ventrally directed apex of the triangular cross-section of the base of the tail forms a kind of keel. This keel extends backward on the ventral side of the tail for a considerable distance as a fairly distinct body, but nothing of the kind has been found on the dorsal surface. The tail of Dr. Coker's specimen, elsewhere referred to, is black and shows a slight ventral keel. This fact has, I believe, not been recorded before.

In the largest Key West specimen the apex of the triangle forms a plainly marked keel on the ventral surface of the root of the tail. This extends in the dried tail in diminishing size backward as far as the tip of the last spine. In this same dried tail on the ventral surface, about the middle of its length, is a very small but plainly perceptible keel. This, however, may possibly be the result of desiccation. In none of these specimens is the tail finned, though we may expect to find an embryonic finfold in the tail of the larval forms.

The table on page 261 records the lengths of the body and tail, absolutely and in relation to each other, for every specimen for which measurements have been given. Ordinarily the tail is said to be 2½ or 3 to 4 times the length of the body (Jordan and Evermann, 1896, and Smith, 1907). The extremes are that the length of tail varies from 1 to 6.2 times body length, the average being 2.9 for 28 specimens, counting in these wide variants. Excluding the extremes, the average tail is 2.7 times the average body in length in 26 specimens. The wide variations recorded in this table are easily explained as follows. It is rather unusual to find a specimen other than a young one with a perfect tail, and the larger and older an eagle ray is the more likely is it to have suffered mutilation in its caudal appendage. Mr. Coles assures me that such has been his experience.

My largest and most perfect specimen from Beaufort had a length of 26.5 inches and a tail measuring but 27.8 inches, the ratio being 1 : 1.05. This tail had plainly suffered amputation, as had the tail of four spines elsewhere referred to. Both were enlarged and bent at the tip, the extreme point being somewhat smaller than the part just anterior to it. Both somewhat resemble fingers amputated beyond the last joint. Only the very young rays have fine slender tails.

My largest Key West specimen, which was a full-grown male, was 3⅔ feet long and possessed a tail 6 feet 10 inches long; the ratio being 1 : 1.9. For so large a ray this one had a very long and slender tail, but about midway of its length there is a very prominent knot, showing that it had been broken but had grown together again. Still further back is a marked bend, showing that it had been injured there also. The tip itself looks as if it had been abbreviated.

Annandale (1910) found the back and tail of a large female *A. narinari* from the Bay of Bengal to be studded with "small star-shaped denticles. On the head these are sufficiently close together to form a regular pavement, while on the tail they have a spinous character." Nothing of the kind has ever been noticed on any of my specimens, not even on the largest, but Jordan and Evermann (1898), in describing *Myliobatis asperrimus* from Panama Bay, say that it has the greater part of the dorsal surface of the body and tail covered with minute stellate prickles.

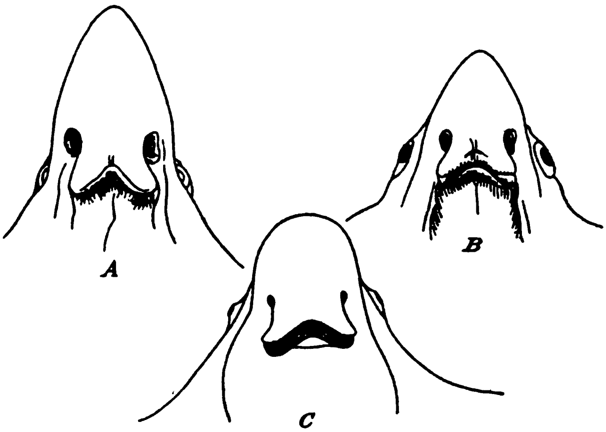
HEAD AND SNOUT.

Jordan and Evermann's figures of *Aëtobatus narinari* are possibly the best we have, but compared with those found in this paper they show certain marked differences in the head region. In their drawing the head seems to stand sharply above the level of the body, but the photographic lateral view (fig. 13, plate VI) shows that this is not true, the body in the head region being slightly thinner than it is further back. In the drawing the lower and outer edges of the spiracles are much more strongly marked than in the photographs. In the living fish there is little or no line of demarcation; the skin continues from the dorsal surface of the body into the spiracle without break, as is attested by the fact that spots are found within its cavity. Various figures (on plate VI) show this admirably.

In the drawing the eyes show very prominently; in the photograph (fig. 1, plate I) as in the five living or just dead and the four preserved specimens examined by the writer, they are not visible from above since they are hidden by the lateral projections of the head in front of the spiracles. In Duméril's drawing from the preserved specimen they are barely visible (fig. 8, plate IV).

In the photograph (fig. 1, plate I) the snout is seen to be pointed and of moderate length. Being below the general level of the body region, and the camera being focused on the mid-dorsal region, the head is not shown to best advantage, being somewhat foreshortened. However, the ventral view (fig. 2, plate I) shows the snout in perfect focus. Here it is seen to be long, rather slender, and distinctly sharp-pointed. It is in marked contrast with text-figure 4, which is Jordan and Evermann's ventral view.

In this connection, it is of interest to contrast with my photographs Annandale's outline figures of the snouts and mouth parts of other eagle rays (text-fig. 6). *A* shows mouth parts and snout of *A. flagellum*, *B* of *A. guttata*, while *C* purports to be a reproduction of Jordan and Evermann's figure, showing these structures in *A. narinari*. However, a comparison of this figure with the original (text-fig. 4) will show how much the artist has erred. If the snout of my figure 2, plate I, were drawn to the same scale, it would be almost as long and slender as *A* in text-figure 6. Figure 25, plate X, is a photographic reproduction of Annandale's elegant figure of *Aëtobatus flagellum*. One's attention is at once called to the notably projecting head and snout, these being strongly marked specific characters for this ray.



TEXT-FIG. 6.—Ventral views of heads of: *A*, *A. flagellum*; *B*, *A. guttata*; *C*, *A. narinari*. After Annandale, 1910.

The structure of the head and snout is not well brought out in figure 1, plate I, but is much clearer in figure 14*A*, plate VI, which is the head only of specimen No. 1 for 1910, elsewhere described. The head is large and prominent, the spiracles especially so, and the eyes are placed laterally on the sides of the head and are invisible. The snout, however, is of special interest, being long and pointed, quite as much so as is the snout of *A. guttata*, *B* in text-figure 6, according to Annandale. The tendency of the cephalic fins to curl up is noticeable in this figure. The white marks on the head are scratches received in handling and transportation. The fish when caught was perfectly normal. Another type of snout is seen in figure 14*B*, plate VI. This is the head only of specimen No. 2 for 1910. This fish had a snout longer and more pointed than any which the writer has yet seen. In these respects it is comparable to Annandale's number *A* in text-figure 6.

An entirely different type of snout is found in figures 15 and 19, plates VI and VIII. These are photographs of the head of Coles's 1909 specimen. The asymmetry of the snout at once challenges attention, as also does its bluntness and its great width of base. The tending to wrinkle of the loose skin covering the cephalic fins is well shown in the former figure. The other spotted ray taken in 1909 also had an asymmetrical snout and cephalic fins which curled up laterally.

The relative positions of the various parts of the head may be more readily understood by reference to figure 13, plate VI, which is a lateral view of the same fish whose head is shown in figures 15 and 19, plates VI and VIII. Clearly brought out are the lateral positions of both eye and spiracle, and the elongated form of the latter with its valve. This valve is hinged on the roof of the opening in such manner that it swings upward, inward, and backward into a recess to open the spiracle. The semilateral position of the gill-slits, especially of the anterior ones, should be noted. These in the ordinary rays are ventrally located, while the eyes and spiracles are dorsal.

A better view of the head and snout is shown in figure 12, plate VI, a photograph of the same fish shown in figure 1, plate I. It has the pectoral hanging down and the throat is supported on a small box to keep the mouth clear. Attention is particularly called to the color of the snout, the spots on the head, the position of the spiracle at the junction of pectoral fin and head, and the eye with its vertical pupil immediately over the mouth. The snout of Coles's specimen No. 1 for 1912 (fig. 9, plate V) does not show very well. However, the spiracle with its spots is well brought out.

These are the only lateral views of the head of *Aëtobatus* known to the writer. In the course of this research, however, a similar view has been found of the head of another eagle ray. In figure 21, plate VII, is shown the head of Miklouho-Maclay and Macleay's spotted ray, *Myliobatis punctatus* (1886). If comparison of this be made with the preceding figures, marked differences will be seen. The snout is long and slender and curiously upturned at the tip. The spiracle bears the same relative position to the pectoral as in *Aëtobatus*, but the eye is considerably further forward, though still over the mouth.

Figure 16, plate VI, is a "head on" view of the ray lying on a table with the throat supported on a small box to keep the under parts of the head clear of the table. The points of interest shown in this figure are the nasal openings, the color on the snout, the spots on the forehead, the eyes placed laterally with the ball just below the line dividing dorsal and ventral surfaces, the semilateral position of gill-slits, and the size and position of the "wings," the pectoral fins. Above all the figure shows how the ray justifies its name, *Aëtobatus*, eagle ray. Another attractive figure is No. 10, plate V, made from one of Mr. Coles's admirable photographs of his ray No. 1, 1912, taken at Cape Lookout. Attention is called to the relative position of spiracles and eyes, to the sink in the head over the brain, to the white streaks under the eyes, to the dark and spotted nose, and to the spots thickly scattered over the head.

Before leaving the subject, it may be well to refer to the structure of the head and snout shown in various other figures reproduced in this article. Duméril's (1861) elegant figures are shown in figure 8, plate IV. Figure *A* is *A. latirostris; B* is *A. narinari;* while *C* is *A. flagellum.* All these are plainly drawn from preserved specimens, as the shrunken parts show. All three have the median cartilage with the lateral cephalic fins very plain. Very interesting is the gradation in length and width of snouts, and the marked descent from forehead to snout level. The snout of Duméril's *A. narinari* is fully as short as that in my photograph, but much wider. The long, pointed head and snout of *A. flagellum* correspond closely to those same structures in Bloch and Schneider's and to Annandale's figures of the same ray, herein reproduced as figures 24 and 25, plate X.

The head in Gunther's (1880) outline sketch (text-fig. 2) is fairly good. The eyes are out of sight, being covered by the forward prolongations of the base of the pectoral fins. The distance between eyes and spiracles is too great. The snout anterior to the forehead region is too short, and the cephalic fins are too small. It is greatly to be regretted that Forster's (1844) figure of his *Raja edentula* was never published. His description tells us that it had a triangular snout, blunt and flattened, cartilaginous, of medium size, and with edges which showed a tendency to curl or roll up.

Russell's (1803) Eel Tenkee (fig. 6, plate III) had the head too mechanically drawn. The snout is very sharply pointed; the eyes are lateral but too prominent, the fontanelle over the brain is too sharply differentiated from the dorsum, when compared with Beaufort and Key West specimens. Russell says that the snout is soft and that it turns up slightly at the point, The head in Euphrasen's (1790) drawing (fig. 5, plate III) is very poor and unsatisfactory. Both eyes and spiracles are dorsal. The snout is too wide, too short, and lacks the cephalic fins.

Marcgrave's (1648) (text-fig. 1) has the spiracle too far behind the base of the pectoral, and the snout too short, blunt, and rounded. The water-color painting (fig. 3, plate II), from which the figure was reproduced, is considerably better done as to snout, eye, and spiracle; while the oil painting (fig. 4, plate II) has eyes dorsal, spiracles invisible, and snout very long and pointed. The former is an excellent figure, the latter very poor.

In the relative size and shape of the head and snout, as in other structures, the spotted eagle ray shows great variations. In Beaufort specimens we have snouts both symmetrical and unsymmetrical. Two had snouts like those shown in figures 15 and 19, plates VI and VIII, while all the others had equilateral snouts more or less sharply pointed. Duméril's *A. latirostris* (fig. 8, plate IV) is possibly a different species. All the Key West specimens had symmetrical snouts fully as pointed as that shown in fig. 14*B*, plate VI.

JAWS AND TEETH.

The "Portugal" quoted by Purchas (1625), who speaks of —"these Rayes some have in their mouth two bones, and break with them the Wilkes," was certainly referring to Myliobatids, mill-toothed rays, and possibly to our particular form.

However, the earliest definite reference to the singular jaws of this fish is found in Marcgrave's original description. It is worthy of repetition here, for, when it is remembered that Marcgrave studied this ray some 270 years ago on the wild coasts of a new and savage world, one must marvel at the care and minute accuracy with which he did his work. He says:

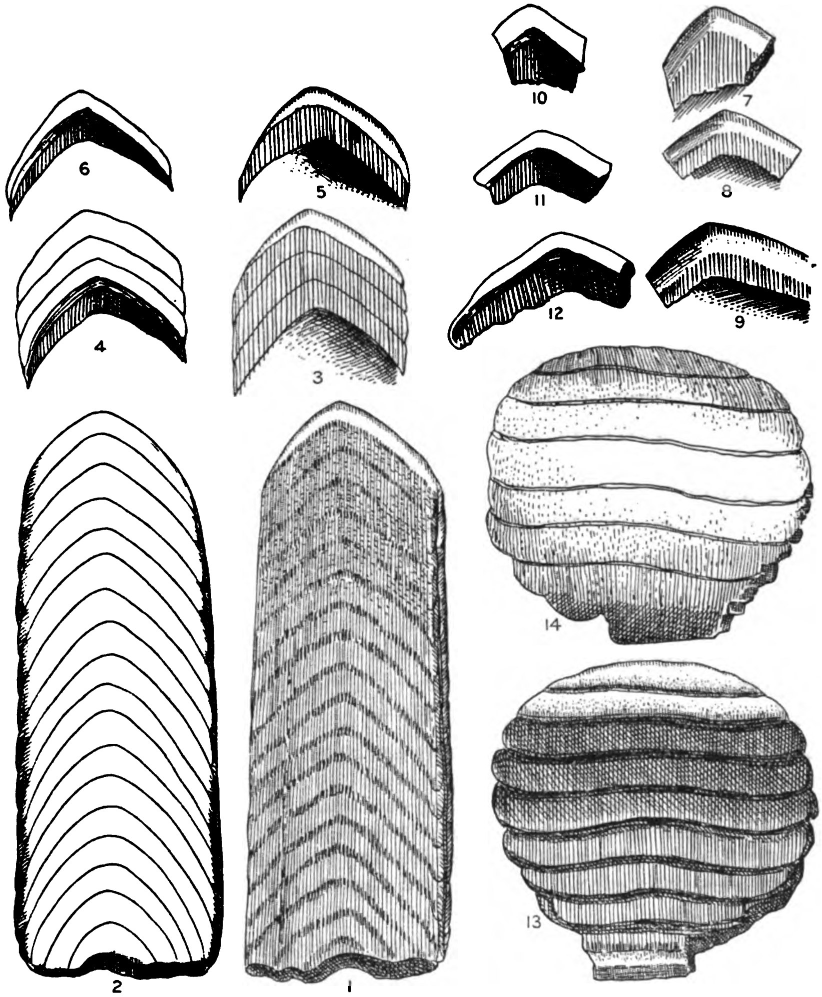
> The mouth is 2½ inches wide, toothless, but having in the place of teeth a lower jaw in the shape of a tongue. This is 4 inches long, 1½ inches wide, and reaches to the external opening of the mouth. Likewise there is an upper jaw placed crosswise, 2 inches long and as many wide. The lower jaw consists of 17 hard white bones having the shape of the letter U and joined firmly to the membranes. Underneath there lie 17 other bones, one under each, of spongy appearance, but not so hard. The upper jaw consists of 14 bones, shaped like the letter J and also joined together by membranes. Likewise there lie above these 14 other bones. Moreover the two jaws are joined to the other bones of the head by membranes [cartilages?].

If the reader will now turn to figures 22 and 23, plate IX, he will see photographs of the remarkable dental armature of this fish. The coincidence may be noted here that the jaws of my 1909 specimen had exactly the number of teeth given by Marcgrave. He speaks of the lower jaw being toothless, but consisting of a tongue-shaped bone 4 inches long, 1½ inches wide, and reaching to the lips. My original notes, made before reading Marcgrave, describe the jaws as being like the slipper-shaped stirrup of a woman's side saddle, the lower jaw being the slipper or bottom part of the stirrup. In illustration see figures 22 and 23, plate IX, of the jaws, and figure 2, plate I, and figure 19, plate VIII, in which the lower jaw is seen projecting from the mouth. Six complete sets of measurements of as many jaws of Beaufort specimens will be given later and four sets for Key West rays.

What Marcgrave meant by his statement that in the upper and under jaws there are other bones, one under each tooth, I am at a loss to understand, unless it be that he refers to the spongy foundation part of each tooth. So Schneider (1801) conjectures when he says that Willughby (whom he seems to have known as a mere copier of Marcgrave) probably refers to the crenate base or some other part subjacent to the teeth for the "under" spoken of in the description. The teeth of this ray are composed of an upper part apparently made of enamel and a lower part seemingly composed of dentine, as will be shown later.

The first man who ever published a figure of the curious jaw structures of *Aëtobatus narinari* was Hans Sloane, whose drawings, from volume XIX of the "Philosophical Transactions," published in the year 1697, are herein reproduced as text-figure 7. Sloane calls this the "tongue" of a flat fish, named *Pastinaca marina*, akin to the Thornback Ray of Great Britain, and found in the waters of Jamaica. He compares this to the lower mandible in man, finds it composed of 19 bones (his figure shows 18) separated by furrows. He confuses top and bottom of the jaw, calling No. 2 the under side when it is the upper, and similarly for No. 1, which is the under side with all the cartilages removed, showing the spongy bases of the teeth previously referred to. Numbers 3 to 12 show very clearly the relation of the enamel and dentine in the individual teeth, and also the shape of the teeth of the lower jaw. Numbers 13 and 14 of this figure are drawn from fossil upper jaws of an Aëtobatid sent Sloane from Maryland. These earliest figures have yet to be improved upon.

The older authors (Willughby, Ruysch, Jonston) either quote Marcgrave or else utterly omit any references to the jaws. Even Euphrasen (1790) contents himself with merely saying: "Mouth below, as in the others of its kind, transverse, with very few and close fitting teeth"; from which we may conclude that his examination was very superficial.



Text-fig. 7.—Teeth of a *Marina pastinaca*, of Jamaica. After Sloane, 1697.

Schneider (Bloch and Schneider, 1801) first quotes Euphrasen as to the teeth, next gives Forster's description (see Lichtenstein, 1844), and then speaks of a figure of the jaws of a *Raja narinari* belonging to Blumenbach in Göttingen. Finally he thus describes a bisected, dried flagellate ray which together with a pair of dried jaws had come into his possession:

> I found in it the same tooth structure as in the free jaws. In the upper jaw there were 8 teeth in the form of the letter ∀ and joined together by membrane; in the lower there were 12, each imitating the letter ζ, all joined together by their crenate bases to the maxilla and occupying its middle part, it being bare on each side.

Next Schneider quotes Willughby (whom he says copies from Marcgrave) and gives the arrangement and kind of teeth correctly for each jaw. This being true, it is inexplicable why he should have attributed the V-shaped teeth to the upper jaw of his specimens, and all the more so since his figure of the under side of the head of *A. flagellum* (the first ever published) shows the long, projecting under jaw with angled teeth (see fig. 24, plate x). Later we shall see that both Agassiz and Owen fell into the same error.

However, Patrick Russell (1803), a physician in the employ of the East India Company, residing at Vizagatapam on the coast of Coromandel, thus speaks of the ocellated ray, *Eel Tenkee* (fig. 6, plate III), which Gunther makes *A. narinari:*

> Jaws dissimilar; the lower arched, narrow, projecting beyond the wider immovable upper jaw; the edges of both are small, without teeth.

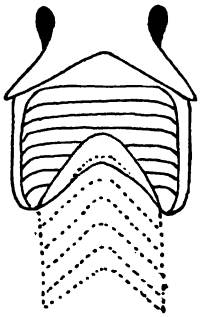
He undoubtedly examined the jaws superficially, as his statement "without teeth" would indicate, but this is probably an echo of Willughby, whose book he had and who in turn merely copied Marcgrave.

Blainville (1816), in establishing the genus *Aëtobatus*, contents himself with merely speaking of the teeth as "broad, smooth, polygonal, united, palatine." However, in 1828 when, without any cause discoverable he changed the name to *Aëtobatis*, he goes further: "Teeth large, smooth, polygonal, united into two plates, one lingual, the other palatine."

The first man after Sloane to study the tooth structure of the spotted sting ray was Ruppell, who in 1835 described, from the Red Sea, an identical form or one very closely related to *A. narinari*. Not only did he accurately describe the ray but he both figured and described the teeth. Text-figure 8 is a reproduction of his drawing of the mouth and jaws of *Myliobatis eeltenkee* (synonym for *A. narinari*). He writes:

> The mouth itself is furnished both above and below with a flattened bony mass, whereof that on the upper jaw is subdivided by 6 cross-furrows into 7 rectangular pieces lying one behind the other. The bony plate of the lower jaw extends in front in a sharp angle; it is smaller than the other and like it is divided by furrows running parallel with the outer edge into 7 equal-sized angular pieces lying one behind another.

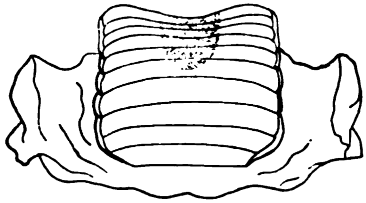
That this jaw was sketched without removal from the body is plain both from the figure (note the nasal flaps and the dotted lines for the lower teeth) and from the short count. This author was evidently unacquainted with Marcgrave's and Sloane's earlier and more thorough work.

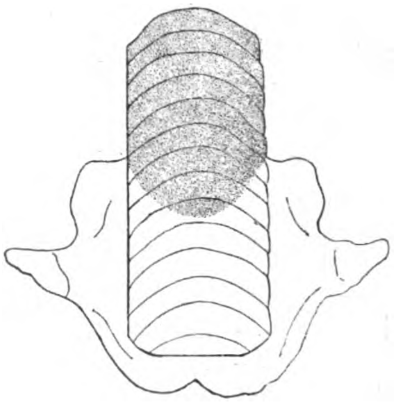


TEXT-FIG. 8.— Jaws of *M. eeltenkee*, from the Red Sea. After Ruppell, 1835.

One year later (1836) Louis Agassiz brought out the third volume of his "Recherches sur les Poissons Fossiles." In this volume he deals with the Placoides, and in order to give them their proper setting he briefly takes up the living forms. Of *Raja narinari* or *Aëtobatis narinari* or *Myliobatis narinari* (he uses all these names) he writes as follows:

It is characterized by a single row of large teeth on each jaw. I have figured it in table D, figures 1 and 2 [here reproduced as text-figs. 9A and 9B]. The teeth of the lower jaw, figure 2 [text-fig. 9A of this paper] are transverse, while those of the upper jaw are more or less arched, figure 1 [here text-fig. 9B].





A. Upper jaw (Tab. D, fig. 2). B. Lower jaw (Tab. D, fig. 1).
TEXT-FIG. 9.—Jaws of *Myliobatis* (*Aëtobatis*) *narinari*, after Agassiz.

It seems unbelievable that Agassiz should have allowed to slip into his work so great an error as the above transposition of the jaws, but he did so and he repeats it in the explanation of the plates. Where he says upper, we should read lower, and vice versa. He next goes into a microscopical examination of the tooth structure, into which we will not follow him here.[1] This occurs on pp. 79 and 80 of the general introduction to his "Poissons Fossiles," volume III. However, on pages 325–326 he gives the following illuminating description of the jaws and their functions.

> This genus (*Aëtobatis*) is characterized by the form of the jaws, of which the lower projects in front, while the upper is much shorter and is squarely cut off. Both are furnished with a single row of transverse teeth without lateral chevrons. The lower jaw is, as in the genus *Myliobatis*, longer than the upper jaw (tab. D, fig. 1) [present text-fig. 9B]. The bone of this jaw is longer than wide. The dental plate, whose surface is almost flat in its whole extent, does not cover all the surface of the jaw. In return, its anterior part projects considerably over the jaw, and as the teeth are arched in front, this only makes the anterior edge more salient. Since all the teeth are parallel with each other, their surface offers the aspect of strips curved and joined, the one to the other. The last tooth alone is transversely cut off. The anterior part of the dental plate, which is lightly shaded in figure 2 of table D [present text-fig. 9A], is used for the rubbing of the two jaws against one another. The upper jaw is much wider than long. The dental plate covers it unlike that of the lower jaw, in that the strips are almost straight and only a little bent at their edges, and thus they surround the anterior border of the jaw in such a manner as to form an arched surface in front of the gullet. This part of the dental plate is used for rubbing against the point of the lower jaw.

By comparing these drawings with the figures of the Beaufort specimens, either the photographs (figs. 22 and 23, plate IX) or the outline drawing (text-fig. 14), it will be seen that in the jaws of my fish the lower teeth are much more sharply angled, especially in front; while in the upper jaw the teeth of Agassiz's ray are much more concave than mine. The grinding surfaces are in both sets of jaws practically the same, covering five teeth in the upper and nine in the lower jaw.

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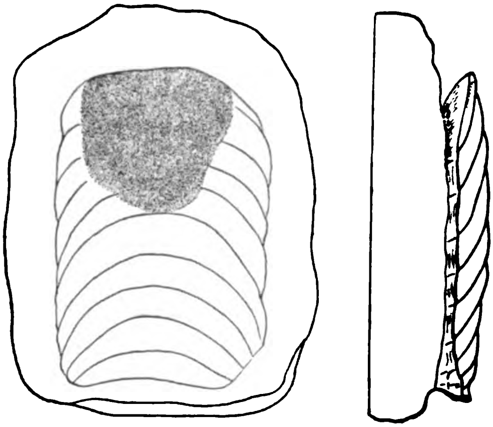
[1] Agassiz notes that the specimen from which these jaws were excised came from the coast of Brazil.

Agassiz records four species of fossil *Aëtobatis* which he had studied, of which he figures only two. Text-figures 10 and 11 are dorsal and lateral views respectively of the lower jaw of *Aëtobatis sulcatus* with ten teeth, the original of which is preserved in the Museum of Paris without indication of its origin.

Müller and Henle (1841), under the heading genus *Aëtobatis*, say:

> The under jaw projects beyond the upper jaw, which has a straight edge. The tooth plates form in each jaw a single row, without lateral teeth, and are in the under jaw bent parallel to the edges of the same. The tooth-plates do not take up the whole width of the jaws.



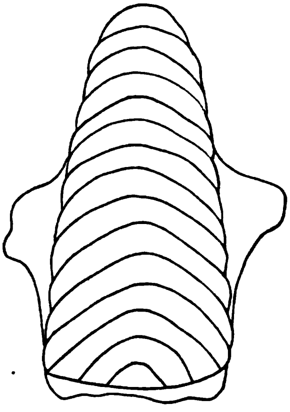
10, Lower jaw. 11, Lateral view of same.

TEXT-FIGS. 10 AND 11.—Jaws of *Aëtobatus sulcatus* (fossil). After Agassiz.

Among the characters for *A. narinari* are:

> The edge of the under jaw and the margins of its pavement teeth present a flat curve which forms in the middle a blunt angle.

Cuvier, in the Atlas to the volume "Poissons" of "Le Regne Animal" (1836–49) on plate 118, figure 4*a*, gives a figure (text-fig. 12) of a lower jaw of which the description reads "Dents de la *Myliobate narinari.*" In the text, however, nothing could be found. The rounded outline of these teeth, as compared with the angled structure of Key West and Beaufort specimens, is very noticeable.



TEXT-FIG. 12.—Lower jaw of *Myliobatis narinari*, after Cuvier.

The celebrated anatomist, Richard Owen (1840–45), in his "Odontography," copies both figures and descriptions (including the error) of *Aëtobatis narinari* from Agassiz. This error was for Agassiz merely typographical, of course, and is, as has been shown, corrected in the text, but the same excuse can not be advanced for Owen. Owen comments upon the absence of the lateral teeth found in the Myliobatids (by which he means Aëtobatids of Müller and Henle), upon the strength of the jaws which support and work such heavy teeth, approaching as they do close to the solidity of bone. However, Owen figures a section through the head of a dried *A. narinari*, showing the projecting lower jaw, calls attention to it, and remarks that it can be used in digging shellfish out of sandy bottoms for food. This figure is so small and the structures so imperfectly shown that it has not seemed worth while to reproduce it here.

Forster, whose collections were made 1772 to 1774, but whose work was first made generally known by Lichtenstein in 1844, thus describes the jaw structures of his *Raja edentula:*

> The lower teeth are of bone joined together like a spatula. These [jaws] are formed of many crescent-shaped bones joined together like tiles by a membrane. These teeth are much longer than the upper ones. The upper teeth are also made up of many bones, much broader than the bones of the lower teeth, and are likewise joined together by a membrane.

Cantor (1849) remarks upon the obtuse angle made by the teeth of the lower jaw and specifically states that the teeth are a greenish-white, being of the same color as the spots and approaching the greenish-gray or greenish-olive of the dorsal surface. The teeth of all Key West and Beaufort specimens examined were white. A possible explanation, however, offers itself for this anomalous color of the teeth of Cantor's fish. While at Tortugas during the summer of 1913, I had occasion to preserve a set of jaws in formalin in a copper tank. When they were taken out some weeks later, they had become impregnated with the copper salts and like Cantor's specimen were of a distinct greenish white. It may be that his specimens had suffered a similar impregnation.

Bleeker (1852) remarks that the upper jaw is as wide as long and has 13 teeth, while the lower with 16 plates is twice as long as wide. Day, in his "Fishes of Malabar" (1865), in giving the characters of the genus *Aëtobatis*, by some strange error speaks of hexagonal teeth with small lateral ones, these being the teeth characters of the family Myliobatidæ. However, in speaking of *A. narinari*, he correctly describes the dentition, noting that the lower teeth are obtusely angled.

Duméril (1865) admirably describes the dental apparatus of the genus, and for *A. narinari* notes that the teeth of the lower jaw form a very open curve. This is in marked contrast to the sharply angled teeth of the Beaufort and Key West jaws and of Ruppell's Red Sea form, but is in complete agreement with Agassiz and with Cuvier (see text-figs. 9B and 12).

Gill (1867) records of the Pacific Coast specimen, *A. laticeps* from San Francisco, that its dental plate had the anterior angle obtusely rounded. However, it must be remembered that Jordan thinks this form a mere variant of *A. narinari.*

In Gunther's diagnosis of the genus (1870), in volume 8 of his "Catalogue," from the study of abundant material, consisting of ten specimens, one set of jaws, and two sets of dental laminæ of *Aëtobatis narinari,* he speaks of a single set of teeth in each jaw, the lower projecting beyond the upper. For the species he writes as follows:

> Teeth of the lower jaw are sometimes angularly bent, sometimes nearly straight. Our series of examples shows clearly that this difference is individual and does not constitute a specific character.

Of two half-grown specimens from the Seychelles, one had the lower teeth angularly bent, the other nearly straight; except for these differences they were perfectly identical. The generic characters of the teeth given in the "Introduction" (1880) are identical with those noted above.

Klunzinger (1871) thus redescribes the jaw structures of the Spotted Ray of the Red Sea:

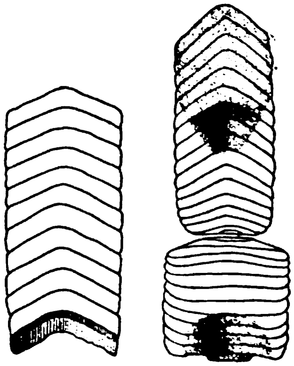
> Upper jaw truncated and broader, but much shorter than the angularly rounded lower jaw which projects from the mouth. The tooth lamellæ of the under jaw form a sometimes pointed, sometimes blunt arched angle in the middle.

In his "Fishes of India" (1878) Day tells us that the teeth of the lower jaw of *A. narinari* may be angularly bent or nearly straight, thus confirming Gunther. He adds, however, that the teeth are greenish yellow. As stated elsewhere the teeth of all Beaufort and Key West specimens examined were white.

Dean (1895), in his "Fishes, Living and Fossil," on page 24, gives a figure (29) with the following legend: "Dental plates of jaw of sting-ray, Trygon (?)." In the text, after referring to the pavement teeth of an ordinary eagle ray (which he also figures), he continues:

> A still more perfect fusion of the dental elements occurs in a ray, closely akin to *Myliobatis;* all lateral elements have been fused, but their metameral sequence has been retained (figure 29).

His figure is plainly that of the jaw of an *Aëtobatus* but unfortunately there is no indication of its source. The commonly accepted idea is that the lateral teeth have disappeared (probably have failed to develop) and that the central teeth have become elongated and arched outwardly. Attention is again called here to Gunther's (1870) series of jaws showing this. Dean's figure is reproduced here as text-figure 13.



TEXT-FIG. 13.—Dental plate of *Trygon*(?), after Dean.

TEXT-FIG. 14.—Teeth of *A. narinari*, from Beaufort, North Carolina.

Jordan and Evermann, in their "Fishes of North and Middle America" (1896) speak of the teeth of the genus as broad, flat, in a single series, upper straight, lower curved, the lower jaw projecting; while for the species they say; "Teeth of the lower jaw straight or more or less angularly bent." Of an Hawaiian specimen, Jenkins (1904) reports the lower teeth to be obtusely angled forward with about five projecting beyond the edge of the upper jaw.

Miranda Ribeiro (1907) thus speaks of the teeth:

> Mouth inferior, with plates of straight prismatic pavement-shaped teeth arranged transversely in parallel rows; truncate in the upper jaw, in the lower the prisms at times folded over to an obtuse angle with points to the front; the plate formed by the latter projects considerably beyond the upper jaw and shows itself over the lower lip.

Compare with this description figures 22 and 23, plate IX. Annandale (1910) finds that the teeth of Indian specimens are transverse and not angularly pointed in the lower jaw.

There are in my possession at this writing six sets of jaws from Beaufort, three of my own taking and three loaned by Mr. Coles. Mine are intact, but Mr. Coles's have the jaws separated and most of the cartilages cut away. Description of these dental apparatuses may not be devoid of interest.

My 1909 specimen was 18 inches long by 26 wide. Its lower jaw (text-fig. 14) is 3¼ inches long by 2 wide, and has 17 teeth of which the anterior 9 are much worn. The upper jaw is 2 inches long by 1¾ wide, and has 13 teeth, of which the 5 anterior forward ones show much signs of wear, the most anterior one having lost the outer third on each side. Owing to the fact that these jaws have been dried flat, *i. e.*, with the upper jaw bent back approximately into the same plane as the lower, I am unable to give the amount of projection of the lower jaw.[1]

Of the 1910 specimens, the first (size 19 by 28½ inches) had a lower jaw 3 inches long and 1 inch wide. It contains 18 teeth, of which 12 show much wear. The upper jaw is 1⅛ inches long by 1⅝ wide, and contains 11 teeth, 5 of which are much worn. The lower jaw projects beyond the upper by 6 teeth or 1 inch. This jaw is viewed from above in figure 23, plate IX.

Specimen No. 2 for 1910, measuring 18½ inches long by 27½ inches wide, being approximately of the same size as the above but having a longer tail, was left as an exhibit in the laboratory and no measurements of its jaws can be given. Ray No. 3, 26½ inches long by 37 wide, was the largest Beaufort specimen I have ever seen. Its lower jaw is 4 inches long and 1⅛ wide, and contains 21 teeth. Of these the 12 forward ones show wear, the most anterior 6 being deeply eroded and the 3 in front having their left edges chipped off. The upper jaw is 2¼ inches long and 1⅝ wide, and contains 15 teeth, of which 6 show hard usage. The lower jaw projects 1⅛ inches or 6 teeth beyond the upper jaw. Figure 22, plate IX, is a lateral view of these jaws showing their relative position when closed.

As noted above, all of Coles's 1910 specimens unfortunately have the jaws separated and cleared of cartilages, and in doing so the soft hinder teeth have been cut away. The size of only one fish can be given. The smallest lower jaw is 3⅝ inches long and 1⅜ wide; it contains 19 teeth, the anterior 8 being badly worn. The upper jaw measures 2 by 1¾ inches; much of the hinder end has been cut off and 4 of its 14 teeth show usage. The second pair measures, for the lower jaw, 3½ by 1⅝ inches. It has 17 teeth remaining, having lost at both ends, for the keen edge on the outermost tooth shows that its predecessor has but lately dropped off. The grinding surface covers the 7 anterior teeth, but at the points of the ninth and tenth teeth there is a slight, irregular depression made by some hard object. The upper jaw of this pair is 2¼ inches long and 1⅞ wide, and of its 13 teeth 5 show much wear.

Coles's largest measured fish was 36 inches long by 69 wide. Its lower jaw, after some considerable abbreviation behind, is still 5¼ inches long, while its width is an even 2 inches. It has 21 teeth remaining, of which the grinding surface extends back over 10, although the next 5 posterior teeth are scarred. The outer right and left thirds of teeth one and two have been broken off. This plate is fully three-eighths of an inch thick, the enamel constituting two-eighths and the dentine one-eighth, but it is very interesting to note that in the outermost and deepest worn tooth the enamel is but one-eighth and the bony portion two-eighths of an inch thick. The upper jaw, which seems to have suffered but little abbreviation, is 3¼ inches long, while its width is 2⅝ inches. It has 16 teeth, 6 of which are in the grinding surface proper, though the next two teeth show rough usage.

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[1] All measurements of jaws are made along the curve and cover or include the teeth only.

It is of course understood that the teeth of this ray, like those of all Elasmobranchs, grow from membrane in the rear and that as they wear out or are broken off in front the growth of those behind keeps the number fairly constant. It must also be made clear that the teeth numbered in the jaws described are the *total* number found by clearing away the overlying fleshy "gum" or membrane. The *apparent* number gotten by counting those visible in the mouth of the fish would be less by some 4 or 5 teeth probably. This fact may be alleged in part proof of Ruppell's (1835) "short" count of the teeth of his ray. He counts 7, but shows 8 teeth in the upper jaw, while in the lower jaw the numbers are 7 and 6 respectively.

The dried jaws of my Key West specimens will now be described. Three of these were rather small, but the fourth was full-grown. All were males.

Ray No. I was 33 inches wide and 24½ long. Its upper jaw is 1⅞ inches long and 1½ wide. It has 17 teeth, of which the forward 6 are deeply worn—practically all the enamel of the outermost tooth having disappeared. The lower jaw measures 3¼ by 1 inches and has 20 teeth; 10 of these show wear, Nos. 6, 7, 8, and 9 are so worn as to be depressed below the general level. Attached to the corners of the outermost tooth are fragments of another, the central and pointed half of which is gone.

Specimen No. II was 32 inches wide by 23 long. Its upper jaw is 1¾ inches long by 1¾ wide. It has 14 teeth, of which the outer 5 show hard usage, a fragment being broken off the right-hand end of the foremost tooth. The lower jaw is 3¼ inches long by 1 wide. It has 20 teeth, of which the 12 outer show wear, this being greatest in the region of Nos. 4 to 8 inclusive, counting from before backwards. The left third of the first tooth is gone.

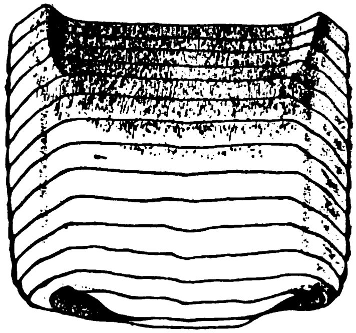
My third specimen for 1913 was 34 inches wide and 24 long. Its teeth are the green ones previously referred to. Its upper jaw, composed of 16 teeth, is 2 inches long by 1½ wide. Its 6 foremost teeth are badly worn, the wear stopping abruptly on the seventh. The outer ends of the front tooth are gone. The lower jaw plate, made up of 21 teeth, is 3⅜ inches long and 1⅛ wide. The grinding surface includes the first 10 teeth, and in teeth 6, 7, 8, and 9 there is a marked depression. The outer tooth has the central half only present, the ends having broken away.

Key West ray No. IV was the largest *Aëtobatus* I have ever had and its jaws are in proportion. It was 62 inches wide and 32 long, and weighed 120 pounds. Its upper jaw-plate measures 3⅛ by 2⅝ inches. Of its 16 teeth, the 4 anterior are deeply worn, the enamel of the outermost one being nearly gone. The lower plate is 6 inches long and 2 wide. It has 21 teeth, of which the anterior 10 show much wear, there being a noticeable depression over Nos. 6, 7, 8, and 9.

These teeth-plates being freed from the jaw cartilages, it is easy to see that the lower plate is gently curved with the convexity upward, while the upper plate is sharply bent with the convexity downward. Thus the anterior edge of the upper plate strikes the lower about one-third of the distance back from its point, and for this reason the lower jaw projects beyond the upper by some 5 or 6 teeth generally, and this also accounts for the worn place in the lower plate as noted above (see figs. 22 and 23, plate IX). In all these Florida specimens the lower tooth-plate projects beyond the lower lip by the width of one tooth, and this tooth is free from the underlying cartilages.

In the ten specimens of lower jaws here described and in the other two examined but never excised from the fish, *all* had the teeth V-shaped, as shown in the photograph and the drawing (fig. 23, plate IX, and text-fig. 14); *none* had the teeth "nearly straight," as described by Gunther (1870), or "teeth of the lower jaw straight or more or less angularly bent," according to Jordan and Evermann (1896), or teeth transverse and not pointed in the lower jaw, as found by Annandale (1910) in specimens from the Bay of Bengal, or teeth obtusely rounded in front as shown by Cuvier and noted by others, especially Miranda Ribeiro (1907).

In my specimens not only does the lower jaw project beyond the upper, but each tooth in it projects beyond the next one behind; thus, seen from above, each tooth overlaps the next one in front like shingles or tiles in a roof. The amount of overlapping is certainly equal to and generally somewhat greater than the width of the tooth. It should be noted here that the soft, pulpy teeth at the hinder end of the jaw are slightly wider than the hard bony ones in front. By reference to the figures of the jaws it will be seen that the ends of the upper teeth are curiously bent backwards, giving each individual tooth the form of a very flat bow (text-fig. 15). Careful measurements of every set of teeth in my possession reveal the interesting fact that the length of the upper teeth measured between the points at which the outer bends begin is in every case exactly equal to the width of the lower jaw-plate of that set. Close inspection of these teeth plates shows that in the region of these bends there is a slight depression transverse to the long axis of each tooth. This forms a shallow, longitudinal groove on each side of the upper jaw-plate, and, as noted above, accurately marks off the width and place of contact of the lower jaw. The worn surface of the



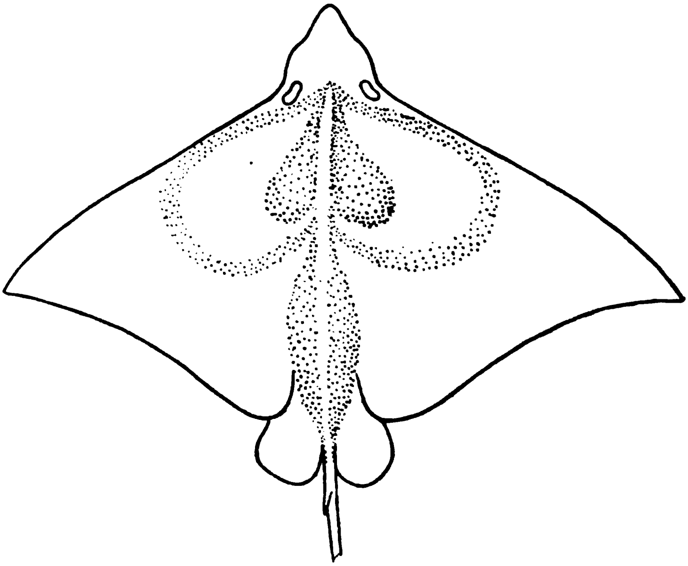
TEXT-FIG. 15.—Upper tooth-plate of an *Aëtobatus narinari*, from Key West, Florida, 1913.

upper jaw is marked off pretty accurately by these grooves, rarely extending out beyond them more than a mere fraction of an inch. The lines formed by these depressions may be seen if looked for on the smaller specimens, but on the large jaw-plate are very plain, being probably one thirty-second of an inch deep. These points have never been noted before. Text-figure 15 is made from the upper jaw of the largest Florida specimen. In front is shown the worn area, and extending backward on each side in the region of the bends of the teeth are faintly stippled areas marking off this depression.

From a consideration of the data above it will be seen that in the teeth, as in all other structures, the spotted eagle ray is evidently subject to very great variations.

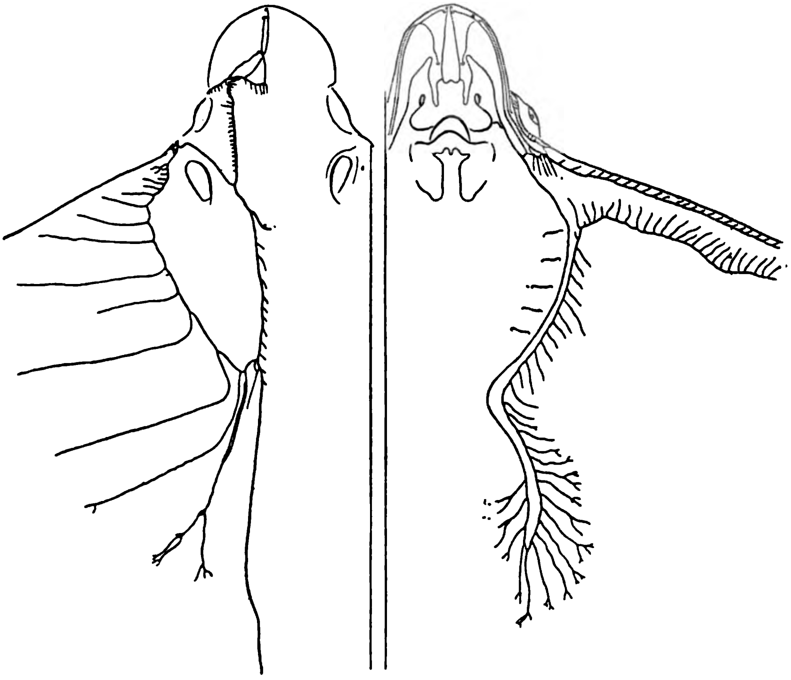
DORSAL SENSORY PORE SYSTEM.

There is now to be described a system of sensory pores on the dorsum of *A. narinari* which has never been noted before, and which I myself overlooked on Beaufort specimens, but found on the frozen Florida rays and also on the live one.



TEXT-FIG. 16.—Dorsal sensory pore system of *A. narinari* from Key West, 1913 (semidiagrammatic).

Beginning back of and between the spiracles and running backward on each side of the median line are fanciful figures made of lines in the flesh, each ending in a black pore. Some distance forward of a line connecting the points of the pectorals, these bands of pores diverge to form two backwardly extending and diverging lobes. However, extending backward between these, on either side of the spinal column, is a band of pores similar to the anterior ones. These widen out over the soft part of the back, dorsal to the abdomen, and further back they nearly converge just in front of the dorsal fin, where they form very distinct patches. From these patches on either side of the dorsal a series of pores extends backward on the sides of the tail, running out to nothing under the spines.



Text-fig. 17.—Lateral-line system of *A. narinari:* A, Dorsal; B, ventral. After Garman.

Beginning at and behind the spiracles a second series of pores extends out along the front edge of each pectoral to about its middle point, then each swings backward, inward, and forward to join the two parallel backwardly going bands just posterior to the lobe-like patches previously referred to. These lines and pores form fanciful figures which run both over and between the spots. So far as my specimens showed, these pores are found rather sparingly on the head, being most abundant on the snout and below the eyes. These pores are presumably homologous with the lateral line pores of Teleosts. Text-figure 16 is a semi-diagrammatic drawing of the dorsal pore-system of *Aëtobatus narinari.* Pores were found on the under side of the body, but not making figures as on the dorsum. The under side of the snout and the central part of the upper lip were so filled with a network of cavities, rather larger than the dorsal pores, as to give the skin over these parts a spongy appearance.

Since this paper went to press, I have chanced upon Garman's memoir (1888–89), "The lateral line system of Selachia and Holocephala." Since his figure and its description are very different from the above, the former is herein reproduced as text-figure 17, A being the dorsal, B the ventral view of the same.

HABITS.

FEEDING HABITS.

Of the spotted sting ray's habits we unfortunately know almost nothing. The fish is too large to be kept in captivity, and its free natural life is such as to preclude anything save scattering observations as to its ways of living.

It is probable that the anonymous "Portugal of Brazil," whom Purchas (1625) quotes in chapter I of volume VII of "His Pilgrims," had reference to our ray when he wrote ". . . . . these Rayes . . . . . have in their mouth two bones, and break with them the Wilkes" whereon they feed. These were undoubtedly pavement-toothed rays, since of all the rays they only live on such fare. But the "two bones" are certainly much more isolated and distinguishable structures in the mouth of an *Aëtobatus* than in that of a *Myliobatis*. Further, our *A. narinari* was common along the coast of Brazil. All these points strengthen the conjecture that *A. narinari* is referred to.

But three of the older writers refer to its feeding habits. Most of them simply copied Marcgrave, while the few who were so fortunate as to examine specimens usually had only preserved material. Furthermore they were systematists, concerned with little else than the characters necessary for classification.

The first of the three is Piso (1648), who in his "De Medicina Brasiliensi," in Book III, bearing the title "De Venenatis & Antidotis," under the sub-heading "Pisces Venenati" writes of *Narinari* that "They are not captured away from the shore. They feed on fishes, for which they lie in ambush and which they capture with the sting of their tail. When they have removed this, they eat them." Here Piso either got his information at second hand or else made a conjecture. Later it will be shown that they live on mollusks alone. However, Piso is correct as to their being shallow-water forms, and it should be noted that this observation of his is the beginning of our knowledge of the habits of the fish.

Sloane (1697) describes the tongue of a ray of Jamaica (plainly *A. narinari*) as made up of 19 bones separated by furrows, and adds that this tongue works against the like bones of the upper jaw to cut, tear, or grind the food; unfortunately he gives no idea of what this food consists. Later, in writing of the same fish, Sloane (1725) says that these rays use their tails "to round their prey to strike them better." In the next paragraph, however, he adds, "They are to be found everywhere in shallow Waters where I was informed they feed on Herbs, Fuci, or Grass." For Sloane's drawing of the teeth of this ray see text-figure 7.

Blainville (1828), speaking of the genus *Raie*, of which he gives *Raies aigles*, *Aëtobatis*, as an example, says:

> The Rays are voracious fishes which feed on other fishes and also on mollusks and crustaceans, which they probably do not catch by swimming, but seize them at the bottom of the seas, in the mud where they have hidden themselves and whence they are able to be dug out by the rays' snouts.

Ruppell (1835) gives a drawing of the jaws (text-fig. 8), the first since Sloane (1725), but it is especially to be regretted that he offers no hint as to the kind of food they are used in grinding.

Agassiz (1836), however, in his figures emphasizes the rough frontal portion of each jaw and explains that this is caused by their rubbing together in feeding; but even he gives no idea of the food of our ray. His figures are reproduced as text-figures 9A and 9B in the present paper.

Owen (1840) figures a longitudinal vertical section through a dried head and jaws of *A. narinari*, comments upon the projecting lower jaw, and conjectures that it "can be used, like a spade, in digging out shellfish, etc., from the sandy bottoms frequented by these rays." Owen is thus the first writer to approximate the food on which this ray lives. He especially calls attention to the strength of the jaws necessary to support such dense and heavy teeth, saying that in density the jaws of this cartilaginous fish approach true bone.

Gunther, in his "Introduction to the Study of Fishes" (1880), remarks of the family Myliobatidæ that the cephalic fins are supposed to be flexible in the living fish and conjectures that they may be used for scooping up food and conveying it from the bottom to the mouth. By implication this statement refers also to the genus *Aëtobatus*. One hardly knows what to think of this. The cephalic fin is soft and the skin-like covering especially so in *narinari;* hence if it is used for the purpose indicated by Gunther, it seems that examination of the living fish ought to show the effects of this hard usage in sand and oyster shells on the snouts. Ordinary sting rays not infrequently are found with bloodshot snouts, but the nine specimens of the spotted sting ray examined by me have shown nothing of the kind. Yet there is no doubt that they feed almost wholly on clams, and Coles (1910) says that they use their snouts for rooting in the sand after the fashion of hogs.

Jordan and Evermann (1896) say of the rays of the family Myliobatidæ that they feed on mollusks, crushing these with their large grinding teeth. Jordan in his "Guide to the Study of Fishes" (1905) makes a similar statement, adding that these fish are destructive to oysters and clams.

Thurston (1894) confirms Jordan by describing the great damage done to the pearl-oyster banks of Ceylon by these rays, the banks being sometimes almost ruined by them. However, he speaks of dissecting an *A. narinari* and finding its stomach full of sea-weed.

The present writer has elsewhere (Gudger 1910) recorded the fact that the food of *A. narinari* seems to consist wholly of clams. Dissections of three specimens in 1911 (Gudger 1912) confirmed this conclusion. However, in not one of the alimentary tracts of the four specimens examined were any fragments of shells to be found. These observations on its food are confirmed in all respects by dissection of the four Florida specimens.

The most extensive and definite observations on the food and feeding habits of this ray have been reported by Coles (1910) in the paper elsewhere repeatedly referred to. He suggests the name "sea-hog" for our ray, on account of its habit of plowing up mud banks and sand-bars with its snout in its search for clams. He has often found it impossible to harpoon rays thus occupied because of the clouded condition of the water. He says further:

> I have known beds, containing many bushels of planted clams, being attacked by schools of these rays and every clam in them destroyed in less than a week; and on several occasions I have had a pile containing a half bushel or more entirely destroyed during a single tide by one or more of these rays. Clams appear to be almost if not entirely the only food of this ray. I have opened more than 50 specimens and have carefully studied the contents of the stomach and have never found that they contain any other food. I am thoroughly convinced that the shell fish consumed by the American people every year are as nothing to the countless thousands of bushels devoured by this ray and its relatives every year.
>
> The muscular development of the jaws of this fish is truly wonderful. I have found in these rays clams which with their shells on must have weighed more than 3 pounds and to crack which a pressure of perhaps a thousand pounds would be required. And I have found in the stomachs of these rays, on a number of occasions, more than a half gallon of clams with the flesh of each clam less broken than the most expert human clam opener could have turned it out; and the writer has often spread out these clams on a clean board and carefully examined them and found that they were absolutely free from any pieces of broken shells.

OFFENSIVE AND DEFENSIVE HABITS.

The spotted sting ray, so far as the writer knows, is an inoffensive fish. The fisherman of Beaufort, notwithstanding the fact that they call this ray the "devil-fish," do not have the fear of it that they do of the common sting ray, *Dasyatis say*, which they familiarly designate "stinger." This is probably due to two facts. First the spotted ray does not, so far as my observation goes, offer so much resistance when taken; and secondly, its spine (or spines), being situated far up on the root of the tail, is not nearly so dangerous as that of the stingaree, which is found about one-third to one-fourth of the distance from the root to the extremity of the tail. Thus the ordinary sting ray can and not infrequently does inflict a wound by lashing out with its tail (I have seen one in the "bunt" of the seine thus drive its sting in the sides of the boat and break off the tip), while, on the other hand, the spotted sting ray, if the object be removed but a few inches from it, can only strike by throwing its whole body. Wounds inflicted by the stingaree are not uncommon and I am acquainted with several Beaufort fishermen who have thus suffered, but the only person whom I know to have been wounded by the spotted ray is Mr. Coles, whose experience will be referred to later.

The commonly accepted idea among fishermen is that this and other sting rays all have poisonous stings, and perfectly reliable men of my acquaintance at Beaufort have described how they have been stung by stingarees, *Dasyatis say*, and how the hand or foot swelled up and what excruciating pain they suffered and how they were disabled for work for a considerable time thereafter. But so far as I can find by dissection and reading no poison gland is ever found in the spotted sting ray or in the stingaree, though there is a groove on the dorsal side of the spines of both rays extending from the point of insertion of the spine to near its tip.

The spine of either ray being closely set with backwardly pointing serrations, it is much easier to cut the spine off and push it through the wound than to pull it out, as Sloane (1725) has pointed out—and such a procedure has been followed in at least one instance known to the writer. The wound is apt to be considerably lacerated, and into it will be carried many pathogenic bacteria imbedded in the slime with which the spine is covered. Furthermore the slime itself, when taken into the blood, may act as a chemical poison. From this it would seem that the explanation of the inflammation is not hard to find. Various modes of treatment are in vogue at Beaufort for the healing of stingaree wounds, but for the most part poultices are used to bring the inflammation to a "head," so that lancing or natural breaking will bring relief.

The only person whom I know to have been wounded by an *Aëtobatus* is Mr. Coles, who in July 1910, while clearing a net, had the misfortune to have a large spotted ray drive its sting 2 inches or more into his leg. He suffered agonies, but having at hand a syringe he thoroughly washed out the wound with a strong solution of formalin. He reports that the pain ceased at once and the wound healed quickly.

The belief in the hurtful and even poisonous properties of the spine of *Aëtobatus narinari* is found scattered throughout the literature from the very beginning. Thus the first European to describe this ray, Claude d'Abbeville in 1614, tells us that its spine is a full foot long and "very dangerous." Marcgrave says nothing on the subject, but Piso (1648 and 1658) writes very interestingly. He tells us that these rays are dwellers in shallow water, where they feed on fishes for which they lie hidden in ambush and which they transfix with the spine. This, however, is erroneous since they feed only on mollusks. Further, our old writer goes on to say that whenever any land or water animal is struck by this spine it receives a virulent poison and the wounded parts suffer great pain, sometimes sufficient to cause paralysis. Whether or not the spine of the dead fish retains its poisonous properties, Piso was unable to say. The antidote for the poison was a plant called Mangue. Piso elsewhere (1658) figures and describes this plant. From the fact that it lives in swamps, and has many branches converted into roots, and further that the figure shows the fruit to consist of long pendant pods acutely terminate at the free ends, there seems to be no doubt that this is the mangrove. Piso further tells us that the natives successfully apply to the wound the ashes of the calcined spine or the split liver of the fish, adding quaintly: "Thus the fish is seen to have in itself the antidote for its poison."

In his later work (1658), in the section dealing with *Narinari*, Piso remarks that many authors have written about the stings of the sting rays, about their poisons and the antidotes therefor, and their hurtful effects on the sound flesh of fishes. These things he says he has verified and has found one of the worst of the sting rays to be *Narinari pinima*, which is the name he gives our ray.

Next, chronologically comes Sloane (1725) whose excellent description of the tail and spines has been quoted on page 274. He adds:

> 'Tis commonly thought this long Tail is useful to the Fish as an offensive or defensive Weapon, wherewith it may lash anything offending it. Or to round their prey to strike them better . . . . . They are eatable: the stings are cut off as soon as they are taken, lest they should hurt unwary People.

Euphrasen tells us (1790) that, since the wound made by the sting is followed by pain and swelling, the inhabitants of St. Bartholomew think it poisonous. Forster (Lichtenstein 1844) is the last author to tell us that the stings are poisonous. He writes:

> The hooked stings contain poison, and for this reason, when the fish is caught, the stings are at once extracted by the inhabitants of Otaheite.

BREEDING HABITS.

Of the manner of reproduction of the Aëtobatids, we have until recently (Coles 1910, 1913) had little definite information. Piso (1658) discourses in general of reproduction in sting rays by the laying of eggs inclosed in horny shells. He ends his long paragraph on this with a few sentences on their stings and poisons and says that chief among such rays is *Narinari pinima*. However, the connection is not close and the inference not clear.

The commonly accepted idea is that all the pavement-toothed rays are viviparous. This belief is probably based on the statement made by Gunther, in his "The Study of Fishes" (1880) and also in his article on Rays in the ninth edition of the Encyclopedia Britannica (1886), that all the Myliobatids are viviparous, but that the young differ much from the adult forms. Waite (1901) quotes a letter from W. A. Haswell, in which the latter states that all the Australian Myliobatids are viviparous. The other Beaufort Myliobatid, *Rhinoptera bonasus*, I have (1912) proved to be viviparous by obtaining (from a specimen only 24 inches wide) two young, measuring 13½ by 8½ inches, rolled up like sheets of paper. Bleeker (1852), by a similar operation on a female *Rhinoptera javanica* of the East Indies, obtained 2 young, 240‴ and 280‴ wide. Couch (1862) in his British Fishes, states that *M. aquila* is oviparous, the eggs being laid in large purses. He figures and describes a young one obtained from such an egg-case; but this must have been a wrong identification. Jordan and Evermann (1896) say that the Myliobatids are ovoviparous, while Smith (1907) affirms that they are viviparous.

Thompson (Jordan and Thompson, 1905) has observed that at the Tortugas these fish not infrequently swim in pairs in long straight lines near the surface. Unfortunately the time of year is not stated. This is probably a pairing play preliminary to copulation. As early as 1810, Risso noted a similar association in the related form *Cephaloptera massena* of Nice. The male was seen for two days swimming around the net in which the female had been taken, "in order without doubt to search for her," and was finally caught in the same net. Blyth (1861) notes of an Indian sting ray, *Trygon imbricatus*, that: "This species is so very often brought in pairs to the bazar, a male and a female, that I can not help suspecting that it lives in pairs, the two being commonly taken together."

The question of viviparity or oviparity in *Aëtobatus narinari* was, however, settled long ago by Klunzinger (1871), who says of the Red Sea *Aëtobatus narinari* that "the foetus measures 12 centimeters." This fact, however, seems to have been generally overlooked. The method of delivery of the young was observed by Mr. Coles in 1910, while fishing at Cape Lookout. After noting that these rays swim near the surface of the water in large schools (one being estimated by him to contain hundreds of individuals) he goes on to state as follows:

> For a number of years my crew and other deep-sea fishermen have been telling me that in giving birth to its young the female ray leaps high in the air as each young is born, but as this leaping seemed so unnecessary I had questioned their tales. However, on about July 15, 1910, I was suddenly called on deck by two of my crew and then I saw a large female *Aëtobatus narinari* leaping high in the air and falling back into the water within 20 fathoms of the yacht. After she had thus leaped several times, I distinctly saw a young one about 6 or 8 inches wide thrown from the body; and after she had again leaped several times without result, another young one was born, and my men told me that two had been born before I came on deck.

While at anchor in the channel off Boca Grande Cay, Florida, in June 1913, numbers of leaping spotted eagle rays were seen, some quite near the boat. I thought that one gave birth to a young one while leaping, but could not be sure.

The explanation of the leaping is probably to be found in the fact that the sudden leap throws the viscera downwards and, aided by the simultaneous contraction of the muscles of the anterior part of the uterus, expels the young. The principle is the same as that by which we drive the last traces of a liquid from an inverted bottle by sudden jerks. Corroborative of this is the fact that whenever a ray is suspended there is a great tendency for the cloacal parts to protrude.

As to how the fœtuses are nourished *in utero* nothing definite was known until Coles published his 1913 paper. Coles states that the uterus of his specimen shown in figures 9, 10, and 11, plate V, was densely lined with large villi 25 mm. in length. These villi secrete a milk on which the embryos are nourished by absorption through the external gills, and later by intake through the spiracles, as the present writer has shown for the other Beaufort rays, *Rhinoptera bonasus*, *Pteroplatea maclura*, and *Dasyatis say* (Gudger, 1910, 1912A, 1913).

However, as early as 1876 Trois had microscopically studied the uterine structures of the related *Myliobatis noctula* of the Mediterranean. He found the walls of its uterus so crowded with villi that the mucosa could not be seen; the number of these, however, decreased towards the mouth of the uterus, which was plicated. These thread-like organs were permeated with blood-vessels, thus enormously increasing the vascularized surface of the uterus. The young were enwrapped by these villi, which, being of great numbers, acted as a feeding organ for the embryos, establishing thus an "efficacious connection between the maternal uterus and the embryo, corresponding to the placenta in *Mustelus acanthias.*"

Since writing the above, the following data have come to light from Cuvier and Duvernoy's "Leçons d'Anatomie Comparée" (1846). They say:

> In the eagle-ray, *Narinari*, there is developed only one oviduct, the left . . . . . of which the first part has a round opening, and is folded lengthwise throughout its whole extent. . . . . The second part . . . . . has walls extremely thick and in great part glandular. The inner surface to a depth of 3 to 4 millimeters is composed of interlacing filaments, forming an irregular mesh. Then comes a compact glandular layer, almost a centimeter thick, in which are distinguishable parallel tubes, running straight from interior to exterior. This glandular part is enveloped in a muscular layer clothed in peritoneum.

Mr. Coles states in his recent paper (1913) that, in the female *A. narinari* which gave birth to the four young above referred to, one uterus was much larger than the other. Dr. Louis Hussakof, of the American Museum of Natural History, at my request kindly examined this specimen, which has been deposited in the Museum, and writes that the left oviduct only is enlarged and villous, "the right being very small in comparison and with the villous layer undeveloped."

The young of Coles's ray above noted, four in number, were delivered rolled up lengthwise, as is common in viviparous rays, but died quickly. The various figures of plate V, but especially No. 11, show these young shortly after birth, while figure 18, plate VII, is a photograph of one of these taken after some months' preservation in formalin or alcohol. On comparing this young with an adult, it will be noted that its snout is shorter, its forehead steeper, its eye and spiracle relatively larger, and especially that its spots are fewer, particularly on the head. This specimen measured 286 mm. across the disk, was 171 mm. long, and the length of its tail was 634 mm. These young were spotted even on the head to some extent, but Annandale's young specimen (not an embryo) of this ray was totally devoid of spots (see fig. 17, plate VII).

In connection with the breeding habits, attention is called to the following table showing the length and width of the fish and the length of the claspers in all the males taken by me:

Beaufort.				Key West.			
Specimen No.	Length, inches.	Width, inches.	Claspers, length of, inches.	Specimen No.	Length, inches.	Width, inches.	Claspers, length of, inches.
No. I....	16	23½	0.5	No. I.....	24½	33	0.8
No. II....	18½	27½	.8	No. II....	23	32	.6
No. III...	26½	37	.9	No. III...	24	34	.6
				No. IV....	44	62	3.8

From this table the conclusion is readily drawn that only the last specimen was sexually mature. Furthermore, comparison of these rays shows that, size for size, males of *Dasyatis say* and *hastata* have much smaller claspers than *Pteroplatea maclura.* Only two other naturalists seem to have noted this interesting point. Ruppell (1835) remarks that these organs are small in his Red Sea specimen, and he therefore concludes that is a young one. Bleeker (1852) says that "the genital appendages are short, conical, and non-valvate." Unfortunately neither author gives the size of his fish. The other common Beaufort Myliobatid, *Rhinoptera bonasus*, likewise has very short claspers. None which I have taken had such appendages as long as an inch. Furthermore Mr. Coles states that all the huge males of *A. narinari* that he has taken at Cape Lookout had claspers shorter than in rays of the same relative size belonging to the genus *Dasyatis*.

HABITAT.

The spotted sting ray, *Aëtobatus narinari*, is cosmopolitan in the tropical and semitropical waters of the world. First found on the coast of Brazil by Abbeville in 1614 and by Marcgrave and Piso in 1648, it has since been reported from the same waters by Agassiz (1836), Müller and Henle (1841), Jordan and Evermann (1898), and lastly by Miranda Ribeiro (1907), 293 years after the French friar above named published his book.

Next it is heard of in the West Indies, where it was taken in Jamaican waters by Sloane (1697 and 1725) and by Browne (1756). Euphrasen's specimen came from St. Bartholomew, one of the lesser Antilles, but he gave as its more extended habitat the West Indies and especially those east of the Caribbean Sea. Forster collected his *Raja edentula* in the South Seas somewhere between 1772 and 1776, but his manuscript was not published until 1844. Thus it came about that, from the time of Abbeville in 1614 till that of Russell in 1804 (when he described his ocellated raja from the Indian Ocean), a space of 190 years, it was thought that this ray was peculiar to the tropical waters of the western Atlantic.

Of later references to its occurrence in Gulf-Caribbean waters, there is no dearth. Henshall (1895) found it on the west coast of Florida, and Thompson (Jordan and Thompson 1905) 10 years later collected and studied it at the Tortugas. While in Porto Rican waters its presence has been recorded by Poey (1881), by Stahl (1883), and lastly by Evermann and Marsh (1900), in their exhaustive study of the fishes of that island. Finally Jordan (1887) and Jordan and Evermann (1896) indicate that it is a common form throughout the West Indies. From personal observation and from inquiry among reliable persons I am assured that it is common throughout the Florida Keys from Tortugas to the mainland.

Drifting north with the Gulf Stream in summer, its presence has been recorded at Beaufort by Yarrow (1877), Jordan and Gilbert (1879), Jordan (1887), Jordan and Evermann (1896), Smith (1907), Gudger (1910, 1912A, 1913), and by Coles (1910, 1913). It should be noted that Coles, fishing at Cape Lookout (12 miles from Beaufort), has seen larger numbers and larger specimens of this ray than any other student of the fish. He captured over 50 specimens and saw great schools estimated to contain hundreds of individuals.

Specimens from the Red Sea have been described by Ruppell (1835), by Müller and Henle (1841), and by Klunzinger (1871). The first reported capture of this fish in the Indian Ocean, and, except for Forster's practically unknown collections from the South Pacific, the first from any waters other than the western Atlantic, was Russell's spotted ray identical with *A. narinari* on the Coromandel coast of India in 1803. Among the specimens on which Müller and Henle (1841) based their description was one or more from Indian waters. Day (1865) first collected it on the Malabar coast, and later (1878) reported it as common in all the northern waters of the Indian Ocean from the Red Sea to the Straits of Malacca, but especially in the estuaries of India. While Gunther (1880) found in the collections of the British Museum two half-grown specimens from the Seychelles, a group of islands north of Madagascar. From this data we see that it is widely scattered throughout the Indian Ocean.

Nevertheless, Annandale, the latest writer on Indian rays, in 1909 expressed the opinion that *A. narinari* is restricted to the Atlantic Ocean, and that the Indian forms are to be classed as *A. flagellum* and *A. guttata.* However, in a later paper (1910), he abandons this conclusion and finds that *A. narinari* occurs in the Indian Ocean in three color varieties. For his illuminating description of these see quotation on page 271.

Found in the Malayan waters as recorded by Cantor (1849) and by Day (1878) as previously noted, Bleeker (1852) says that it is common throughout the whole of the East Indian Archipelago, as his lists of fishes for a dozen islands show. However, for the Australian coast but one record has been found. J. Douglas-Ogilby (1886) notes its capture at Cape Hawke, New South Wales.

It is in the Pacific, as is to be expected, that we find *Aëtobatus narinari* most widely distributed. If it is agreed that Quoy and Gaimard's 5-spined ray is a synonym of our fish, then the first capture of *A. narinari* is reported from Guam, one of the Ladrones, in 1824. It is true, however, that Schneider (Bloch and Schneider 1801) had quoted from Forster's manuscript that the latter had taken and described our ray under the name *Raja edentula* at Otaheite, one of the Paumotu group, in the years 1772–1774.

More captures of this ray have been reported from the Sandwich Islands than from any other group in the Pacific. In 1858, Agassiz proposed the name *Goniobatis meleagris* for a specimen sent him from this region. The name was never adopted and the specimen seems to have been lost. It is presumed that this ray is to be identified as *A. narinari*, since no other ray of the kind has ever been collected there, and Jenkins (1904) and Jordan and Evermann (1905) so agree. In 1901, Steindachner identified as *A. narinari* a spotted ray collected by Schauinsland in 1896–97 at Laysan, one of the extreme northwestern islands of the group. On the taking over of these islands by the United States, their aquatic resources were admirably worked up by the United States Bureau of Fisheries. Fishes are reported on in two papers. Jenkins (1904) lists our ray (making it a synonym for *Goniobatis meleagris*) as common at Honolulu, being for sale in the market, while Jordan and Evermann (1905) procured it at both Honolulu and Hilo.

Elsewhere in the Pacific, Jordan and Seale (1907) have taken it at Cavite, Philippine Islands, and Gunther has described a young male from Samoa preserved in the Museum Godeffroy at Hamburg. In the same article he notes that there is on deposit in the British Museum a pair of large tooth plates collected in the Solomon Islands by Woodford.

In 1867 Gill received from San Francisco a spotted ray from an unknown habitat; to which he gave the name *A. laticeps.* Jordan (1895) finds rays all along the Pacific coast from the Gulf of California to Panama practically identical with Gill's ray and with West Indian specimens of *A. narinari.* Later (Jordan and Evermann 1898) he advises that Gill's specific name be dropped and the ray be considered as *A. narinari.*

Gilbert and Starks (1904) report *A. narinari* as frequently seen in the Bay of Panama and note that the specimens they examined were in no particulars different from those described by Jordan from Sinaloa (1895).

From the above data it will be seen that the habitat of this ray is coexistent with the tropical and semitropical waters which encircle the globe.

ECONOMIC VALUE.

Marcgrave's statement that the flesh of *A. narinari* has a good flavor and is sufficient (in the case of his or some other large specimen) to feed 40 men indicates that this ray among others was eaten in Brazil even at that early day. Sloane (1725) declares it to be eatable, and Browne (1756) says it was well liked by the Jamaicans, while Forster (the specific name of whose ray is *edentula*) some fifty years later found it "not the last fish" at the feasts of the Otaheitans. Further Euphrasen (1790) affirms that its flesh is white and, when properly prepared, that it is equal to that of the best fish eaten in Sweden, from which we may conclude that it was habitually eaten in the Lesser Antilles.

On this subject Bleeker (1852) writes: "The flesh of *Aëtobatis narinari* is of a rather dark bluish color and in Batavia, especially among the Chinese, it is much sought for and commands a high price." Day (1878) notes that this ray is eaten by the natives of Hindustan. Jenkins (1904) found it for sale in the market at Honolulu, from which we may infer that it is habitually eaten in the Sandwich Islands. Probably fuller investigations will show that it is a common article of food throughout the Pacific.

Since great numbers of other and more edible fishes are to be had for the catching or are for sale at exceedingly low prices, neither *A. narinari* nor any of the other rays found at Beaufort or Key West are used for food there. However, it is stated by some sailors and deep-sea fishermen, that the pectoral fins of the rays (kinds not specified) are, when properly prepared, excellent for the table. As to this, the writer is unable to speak from experience, but he has eaten the fins of the common southern stingaree, *Dasyatis hastata*, and found them very palatable.

GENERA AND SPECIES OF ANGLE-TOOTHED RAYS.

The observations of the present writer having been restricted to one form, *Aëtobatus narinari*, and that in but two localities, he hesitates to go into this subject further than to collect here the data brought together in the historical part of this research. It is certain that the fish is subject to great variation, and it is possible that the great differences, especially in color and in the presence or absence of spots on various parts of the body, are, as Gunther (1870) found for the teeth, varietal and not specific. If many specimens from the various tropical waters could be collected and compared by such taxonomists as Gill, Jordan, Evermann, Boulenger, Smith, or Regan, it might be possible to reconcile the many discrepancies revealed in the various descriptions quoted in this paper. Descriptions are at best very unsatisfactory, while figures, being for the most part made from (more or less badly) preserved specimens, are but little less so. What is needed is to have these various rays side by side that critical examination and careful measurements of minute details of structure might be made. But these fishes are so large and unwieldy, and their preservation for these reasons so difficult, that such an examination, however much to be wished for, is of the very distant future.

There are, however, at least two and possibly three or four well-marked species which seem to have gained places for themselves in ichthyological literature. But at least one generic and several specific names have been given which do not seem to have had permanence. One species has been pretty firmly established, but is attributed to an author who merely copied from another copier. Other Myliobatids have by some been made synonymous with *A. narinari*, when they belong to entirely different genera. The facts will be herein presented and the reader may form his own conclusions independently of these expressed by the present writer.

AËTOBATUS NARINARI.

GENUINE SYNONYMS.

The species known longest and best is *Aëtobatus narinari*, the history of whose nomenclature will now be briefly sketched. The name *narinari* (in its various spellings) appears to have been a Brazilian or Indian word meaning sting ray. It seems to have been first applied to the ray bearing that name by Abbeville in 1614, but the name and the ray were together first given a place in scientific literature by Marcgrave in 1648. Euphrasen (1790) first designated it binomially as *Raja narinari*, wisely retaining the native name, and to him is due the credit for having assigned the specific designation. For the specific characters see descriptions by Marcgrave and Euphrasen, pages 246 and 249.

However proper the specific term, Euphrasen's generic name was entirely too indefinite, so Blainville in 1816 established the genus *Aëtobatus*, with the following characters:

Body fleshy with pectoral fins shaped like an eagle's wings; head thick, not beaked (*non rostrata*), furnished with a simple appendage in front; eyes lateral; teeth broad, smooth, polygonal, united, palatine; pectoral fins pointed; anterior margin convex, posterior concave; pelvic fins small, round, whole; dorsal fin single on the root of a tail at times extremely long, flagelliform, armed with a serrate spine, without fin at extremity.

Following this is a list of species, ten in all, of which *narinari* is one.

In 1828, Blainville, in "Faune Française" under the heading "Poissons Cartilagineux," without assigning any reason whatever therefor, writes the generic name *Aëtobatis* and repeats almost verbatim the generic characters given above. The chief difference is that the teeth are now described as "large, smooth, polygonal, united into two plates, one lingual, the other palatine," but in neither set of characters are the teeth of the lower plate described as bent or angled with the projection forward. The generic term *Aëtobatus*, having been assigned with definite and correctly designating characters twelve years previously, is the correct name for the genus and is accordingly so used throughout this paper. It should be noted here that *narinari* is not named as a species under this new genus *Aëtobatis*.

Müller and Henle (1841), in their epoch-making "Systematische Beschreibung der Plagiostomen," once and for all gave our ray a definite place in ichthyological literature as *Aëtobatis narinari*. Their description is elsewhere (page 251) given and need not be repeated here, but it should be noted that, among the generic characters, they say of the teeth that the under jaw projects beyond the upper jaw, which has a straight edge, while the under jaw has the teeth bent parallel to the edge of the same; the teeth plates in each jaw form a single row without lateral teeth and do not occupy the whole breadth of the jaw, while for *A. narinari* it is stated that the lower teeth are in the form of a flat arch (*flache Bogen*). They are the first authors to base their description on abundant material, since they had at hand 12 specimens, 6 dry and as many in alcohol, from such widely different regions as the waters of Brazil, the Red Sea, and the East Indies. The great pity is that, not knowing of Blainville's early name, they should have assigned the later generic term which has unfortunately been used down to the present time.

In working up the synonymy of *Aëtobatus narinari*, it is now necessary to return and to identify certain rays described in the interval between Euphrasen's assigning the specific name and Müller and Henle's firmly establishing our ray as a distinct form.

Schneider (Bloch and Schneider 1801) adopted Euphrasen's nomenclature, *Raja narinari*, and although he copied Euphrasen, Forster, and others, he expressly says that he had a hitherto undescribed specimen. Russell's (1803) *Raja ocellata*, Eel Tenkee of the natives, is plainly an *A. narinari*, as figure 6, plate III, shows. So likewise is Raffles's (1830) *Myliobatis narinari*, which is expressly identified by Bennett with *Raja narinari* Euphrasen and with Eel Tenkee Russell and is thought (erroneously) to be the same as Shaw's *Raja guttata*.

In 1835, Ruppell described his *Myliobatis eeltenkee* from the Red Sea. This ray is, as his figure of the teeth (text-fig. 8) shows, an *Aëtobatus*, and his description as surely makes it *narinari*. Cuvier (1817) gives in the atlas of the volume "Poissons" of his "Regne Animal" a figure (see text-fig. 12) of the teeth of *Myliobatis narinari*, but his text contains no reference whatever to it.

As noted elsewhere, Forster collected in the South Seas in 1772–74 a ray which he called *Raja edentula*. His manuscript, published in 1844, contains a careful description which plainly makes his ray identical with the fish being studied. It has, however, been generally overlooked by authors. Blainville (1816) possibly refers to it when he gives *fosteri* as one of his ten species of *Aëtobatus*. Cuvier, in "Regne Animal" (edition of 1817), lists *Raja narinari*, quoting Marcgrave's *narinari* as a synonym. In a later edition of the same work he gives the name *Myliobatis narinari*. In neither case does he give any description. It should be noted, however, that the earlier reference is in a footnote to the genus *Myliobatis*.

Notwithstanding the lack of spots on the head and anterior edges of the pectorals, there seems to be little doubt that Cantor's (1849) *Stoasodon* (porch or projecting tooth) *narinari* is a synonym of our fish. Indeed his giving it the specific name indicates this. Why he introduced a new generic term is unknown to the writer. Following the lead of Müller and Henle, Bleeker (1852) uses *Aëtobatis narinari*, as does Day (1865), though Day's ray lacked the spots on the head.

Five years later Gunther (1870), from the wealth of material in the British Museum, consisting of ten specimens, two multi-spined tails, one set of large jaws, and two sets of excised teeth, made one species, *Aëtobatis narinari*, and this notwithstanding the fact that the "teeth of the lower jaw are sometimes angularly bent, sometimes nearly straight." Gunther declares that these form such a series that he is satisfied that the differences are entirely individual and in no wise specific. Further he makes all the preceding forms (except *Raja edentula*, which seems to have been unknown to him) synonymous with *Aëtobatis narinari*.[1] This decision of this great systematist, based upon the most abundant material since Müller and Henle's, and resulting in the adoption of their nomenclature and its repetition in his "Guide to the Study of Fishes," authoritatively fixed the name until this day.

Accordingly Klunzinger (1871) named his Red Sea ray *Aëtobatis narinari*, though it lacked the spots on the head. Day (1878) also uses the name for Indian Ocean rays, though they also are devoid of cephalic spots. Jordan and Gilbert (1882) prefer this name, as does Douglas-Ogilby (1886). Henshall (1895), however, reverts to Cantor's nomenclature, *Stoasodon narinari*, for a specimen from the West Coast of Florida.

Jordan (1895) uses for the Pacific coast form the name *Aëtobatus narinari*, which the present writer believes to be correct, while Jordan and Evermann (1896) prefer the same termination for all American rays of this kind. So does Steindachner (1901). Moreover, Evermann and Marsh (1900) and Jenkins (1904) write it *-tus*, as do Gilbert and Starks (1904). Nevertheless, one year later Jordan and Evermann (1905) and Jordan and Thompson (1905) again take up Cantor's generic term *Stoasodon*, retaining *narinari* as the specific name. However, in the same year was published Jordan's great "Guide to the Study of Fishes," in which the name is written *-tus*, while two years later he and Seale name the Philippine form *Stoasodon*.

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[1] Among these are such definite species as the ray called *Raja flagellum* by Bloch and Schneider and *Aëtobatis flagellum* by Müller and Henle, as well as such doubtful ones as *Raja guttata* Shaw, *Raja quinqueaculata* Quoy and Gaimard, to say nothing of such a form as *Goniobatis* (correctly *Myliobatis*) *macroptera* McClelland, which is not even an Aëtobatine. All the data available will be given for these forms in the next few pages.

Miranda Ribeiro (1907), who, if one may judge by his table of synonyms, has gone into the question of synonymy more carefully than any one else, uses the correct name *Aëtobatus*, referring it directly to Blainville's paper of 1816. This name is also used by Coles (1910, 1913). Not so, however, Annandale (1909–1910) and Gunther (1910), since they retain the Müller and Henle terminology. However, all these names seem to refer to the same spotted eagle ray, which, if there is any virtue in priority, must be designated *Aëtobatus narinari*.

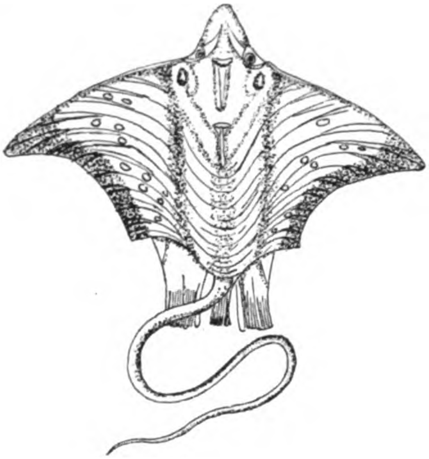
DOUBTFUL SYNONYMS.

It is now necessary to take up the descriptions of certain rays the names of which have been declared by certain authors to be synonymous with the ray originally named by Marcgrave, *Narinari*, but about the correctness of which much doubt exists.

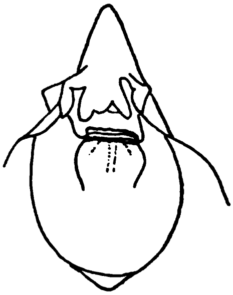
First of these is *Raja guttata*, which is ordinarily ascribed to Shaw, but which was first described by Bloch and Schneider in 1801 in the following words: "Body ashy-gray, black, guttated and spotted, head heart-shaped, tail finned and twice as long as the body." This ray they make identical with Marcgrave's Brazilian ray *Jabebireba* or *Jabebirete*, which, however, is not an eagle ray at all.

Raja guttata, the spotted ray of Shaw (1804), is by many authors identified as *A. narinari*, and Shaw himself says that it is identical with Marcgrave's *Narinari* and with Russell's *Eel Tenkee*. His figure is reproduced herein as figure 20, plate VIII, and his description may be found on page 313 of this paper. Inspection of this figure and comparison with that which forms the frontispiece to this paper, and with any other figure which has been identified in this article with *A. narinari*, will, I believe, show that no allowance for bad drawing can account for the great discrepancies. Later I shall show that this figure is not original with Shaw, but that he copied a mirror image of Lacépède's figure labeled "La Raie Aigle"; and that Lacépède in turn copied an unpublished figure of Commerson's of an eagle ray which he had figured from Madagascar waters. With these facts before him the present writer rejects *Raja guttata* Shaw as a synonym for *A. narinari*.

It is possible that Quoy and Gaimard's (1824) *Raja quinqueaculata* may, as Jordan and Evermann (1896) and many other taxonomists think, be identified with *A. narinari*. The points in favor of such identity are: (1) the elongated snout curved at the tip; (2) body above dark brown in color and sprinkled with round blue spots; (3) tail, which "appears to have been cut off by accident," with a dorsal fin and five spines very similar to those found on my best Beaufort specimen. Their figure of the fish was lost, and, what is most of all to be regretted, they omit any description of those structures, the teeth, which would have forever settled the question of identification. At first the present writer was inclined to admit the identity, but further study has brought out certain objections.



TEXT-FIG. 18.—*Myliobatis macroptera*, after McClelland, India.



TEXT-FIG. 19.—Ventral view of head region of same.

Quoy and Gaimard preserved the tail and deposited it in the Museum (of Paris?) and in their plates give a figure of it. A photograph of this drawing and of my 4-spined Beaufort specimen is shown in figure 7, plate IV. It is very noticeable that the large white spot found on the upper and back part of the dorsal of *A. narinari* is absent from that of the 5-spined ray. In all the dorsals of both Beaufort and Key West specimens examined by me, it has been present save in one only, and it a badly preserved one. This objection alone is, however, not of sufficient weight to negative the identity, but in the course of this research the present writer chanced upon a description by Miklouho-Maclay and Macleay (1886) of a spotted ray, *Myliobatis punctatus*, from the Admiralty and Hermit Islands. This ray also had: (1) an elongated snout turned up at the tip; (2) the upper surface (greenish-gray) dotted with (irregularly scattered dirty-white) spots; (3) a short tail with a dorsal followed by two serrated spines. So far the descriptions are quite parallel, and the figures of this latter ray (No. 21, plate VIII) might well be taken for the former, but these authors go on to describe what the others do not, the teeth which are in "many longitudinal rows." To the present writer it seems quite as easy to identify the 5-spined ray as *Myliobatis punctatus* as *A. narinari*, and because of these doubts he prefers to omit it from his table of synonyms.

Jordan and Evermann (1896), probably quoting Gunther (1870), include among their synonyms *Goniobatis macroptera* McClelland. On looking up McClelland's paper (1840), one finds that he describes and figures, under the name *Myliobatis macroptera*, an eagle ray from the Indian Ocean. Text-figures 18 and 19 are reproductions of his drawings, the latter of the under side of the head. If the figure be inspected carefully, it will be seen that the dorsally placed eyes, the short, round-pointed pectorals forming posteriorly a rounded projection where the tail joins the body, the broad-based, square-ended ventrals, which McClelland says equal one-third the entire length of the body, the short tail, the roughly parallel lines extending across the disk and bent backwards over the body proper, and the few scattered spots on the pectorals only are not found in any figure or description of *A. narinari* published by any other writer. Then if text-figure 19 be studied, it will be seen that the lower jaw is square-cut instead of angled outwards. Further McClelland compares his ray with *M. maculatus*, notes that it has rounded ventrals, whereas his ray has angular fins, and ends by declaring that his ray is an entirely new form. These points clearly show that *Myliobatis macroptera* can by no means be considered as identical with *A. narinari*.

In a verbal communication to the Boston Society of Natural History, June 16, 1858, Agassiz (1859) proposed, on the basis of a specimen sent from the Sandwich Islands, a new genus, *Goniobatis*, for angle-toothed rays having the palate broadest behind and with plates obtusely angular having the rounded edges forward. This description exactly fits Cuvier's drawing of the teeth of *Myliobatis narinari* (see text-fig. 12). For the specific name, Agassiz used the term *meleagris* (guinea fowl), probably in allusion to the spots. Neither term seems ever to have been used, and Jenkins (1904) considers the name synonymous with *A. narinari*. This is probably correct, but as the fish, so far as the present writer knows, was never carefully described, we can not be absolutely sure. Agassiz also proposed that the *A. flagellum* of the Indian Ocean be renamed *Goniobatis flagellum*, but his suggestion was never taken up.

In 1867, Dr. Theodore N. Gill described, under the name *Aëtobatis laticeps*, a spotted ray sent from San Francisco, but of unknown habitat. As the specific name implies, the head was wide and the snout obtusely rounded. Jordan and Evermann (1896) assign it a place in their "Fishes of the North and Middle America" (vol. I, page 88) and give as its habitat the west coast of Mexico from the Gulf of California to Panama, but mark it as doubtfully separated from *A. narinari*. Gill himself notes that it is closely related to this form and also to *A. latirostris* A. Duméril, and probably not far from Agassiz's *Goniobatis meleagris*. In his "Fishes of Sinaloa" (1895) Jordan states, on the authority of Evermann, that this west coast supposed *A. laticeps* is identical with *A. narinari;* while later (1898), in volume III of the "Fishes," etc., he proposes to omit *A. laticeps* as a species on the ground that no essential differences between it and *A. narinari* can be found.

AËTOBATUS FLAGELLUM.

This ray is the second of the two definitely established species. Under the name *Raja flagellum*, it was first described and figured by Bloch and Schneider (1801). They give the characters as follows: Body width equals twice the length, head and pectoral fins pointed, dorsal fin short, one or two spines serrated on both sides at the base of the whip-lash-like tail, which is four times longer than the body. Habitat: Indian Sea on the coast of Coromandel. A photographic copy of their colored drawing is given as figure 24, plate X. This unfortunately does not show the parallel striations running from front to back across the pectoral fins. It must be noted that in their diagnosis of the characters they say nothing of the teeth, but in the smaller figure, showing the under parts of the head, the projecting lower jaw with its rounded tooth leaves no doubt as to these structures.[1]

Blainville (1816) after giving the characters of the genus *Aëtobatus* (see page 307) enumerates *flagellum* as one of the species under this genus.

Müller and Henle (1841), after examining 21 specimens from India and the Red Sea, established the form *Aëtobatis flagellum*, giving as a synonym *Raja flagellum* Bloch and Schneider. Among its distinctive characters are: Snout wide at base, small, and only about three times as long as the nasal lobes; teeth sharply rounded; no spots; disk above dark violet or bronzy or coppery, white below. Richardson (1846) lists this ray in his "Fishes of China and Japan," but does nothing more than refer to Müller and Henle and to Bloch and Schneider. Likewise Blyth (1861) contents himself with recording it in his "Cartilaginous Fishes of Lower Bengal," but gives no information whatever about it.[2]

The next reference to *A. flagellum*, which has been chanced upon is from A. Duméril (1865). In this paper is a fine plate showing the head of what he calls *A. fouet* (*A. flagellum* Müller and Henle). This elegant plate is reproduced as figure 8, plate IV, of this paper, and *C* is the head referred to. Attention is called to the extremely long head and snout, the latter being very slender and narrow, to the lateral eyes, to the spiracles with their backwardly prolonged depressions, and to the complete absence of spots. It is greatly to be regretted that Duméril gives us no data as to the fish from which the figure was made.

Earlier in this paper (page 309) it has been noted that Gunther (1870) makes *R. flagellum* Bloch and Schneider a synonym of *A. narinari*. This is a palpable error which curiously enough has been repeated by Jordan and Evermann (1896), who probably copied Gunther. The facts are as follows: On page 361 of Bloch and Schneider (1801), under genus *Raja*, No. 10 is *R. flagellum;* lower on the same page, No. 11 is *R. narinari*.

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[1] In his 'Analecten für Vergleichende Anatomie," sammlung I, published at Bonn in 1835, A. F. J. C. Mayer describes *Raja fasciata* Mas." This ray was 6 inches long with a 35-inch tail. Of it he says: "Both the upper and the lower jaw is each overlaid with a tooth-plate consisting of about seven horse-shoe shaped lamellæ. . . . The side fins are striped and are cut in a half-moon shape." This might lead one to think that he had an eagle ray like Bloch and Schneider's *Raja flagellum*, but he says further, "Head square (viereckig)."

[2] Agassiz's (1859) attempt to rename this ray *Goniobatis flagellum* has been previously referred to.

Having made no careful search for references to *A. flagellum*, it is quite likely that I have overlooked a number. However, the only other one which has been found is in Annandale's "Batoidei of the Indian Ocean" (1909). This author considers this ray rare in the upper part of the Bay of Bengal, since he has seen but two examples and a head. However, he gives an elegant figure, reproduced herein as figure 25, plate x. Especial attention is called to the extreme length of the head and snout, to the lateral eyes, and to the widely spread ventrals with their claspers free only at their very extremities, as in young Beaufort and Key West specimens.

Text-figure 6 is also reproduced from Annandale. *A* in this is a ventral view of head and mouth parts of *A. flagellum*. The lower jaw in this ray does not seem to project so much as in the other Aëtobatines, notably in *A. narinari*, as shown in figure 1, plate I. *B* and *C* are ventral views of the heads of *A. guttata* and *A. narinari* respectively. The latter purports to have been copied from Jordan and Evermann (1898), but as has been shown elsewhere is very defective. The former ray will now be briefly discussed.

AËTOBATUS GUTTATA.

The history of this ray, the third of the definitely established species, is very obscure, but the facts seem to be as follows: Under the name *Raja guttata* it was first described by Bloch and Schneider in 1801. Their brief and imperfect description may be found on page 309 of this paper. The species seems to have been given a definite place in ichthyological literature by Shaw (1804), whose description reads as follows:

> Greatly allied to *Raja aquila* in appearance, but with a more produced head or snout; color above deep cinereous, pretty thickly marked with small, round, white or whitish spots; tail fins and spines placed nearer the body than in the preceding [*Raja aquila* with an ordinary sting ray (*Dasyatis*) tail and spine], of which, however, it has sometimes been considered a variety rather than as truly distinct; a native of the Indian and African seas; observed by Commerson about the coasts of Madagascar, by Dr. Russel about those of Coromandel, and long ago by Marcgrave about those of Brazil.

Shaw gives a figure of this ray, which on investigation I find to be a copy of Lacépède's (1798) drawing of *La Raie Aigle*, figure 2, plate 6, differing from it only in that the tail is shown bent to the left side, *i. e.*, it is a mirror image of Lacépède's figure. However, one wades through the mass of Lacépède's verbiage about an eagle ray found in the Mediterranean without finding any reference to the figure bearing the same title until in the concluding paragraphs the real facts appear.

It seems that there had fallen into Lacépède's hands certain unpublished manuscripts and drawings of the naturalist Commerson. Among these were the figure and description of an eagle ray which he had found in the waters around Madagascar and the Isles of France. This figure Lacépède published and labeled *La Raie Aigle;* Shaw in turn appropriated and published it as *Raja guttata*, without giving Lacépède any credit whatever, and merely mentioning Commerson, as has been shown. Shaw identifies his *Raja guttata* with Russell's *Eel Tenkee* and Marcgrave's *Narinari*, but (as has been shown elsewhere) this is probably incorrect. We are not even sure that it is an *Aëtobatus*, since the teeth are not described. Figure 20, plate VIII, is a photograph of this Shaw-Lacépède-Commerson figure.

The only other reference to this ray which the writer has found is in Annandale's "Batoidei of the Indian Ocean" (1909). This author says that it is very common in the Bay of Bengal. From his three (two young, one mature) specimens, Annandale thus describes its color:

> Dorsal surface of young of a uniform dark slate-gray, without trace of spots. The spots of the disk on the adult are confined to the posterior half. They are of a bluish tint and are edged with a faint greenish halo. Their size varies considerably. The ground color of the back of the adult has, in fresh specimens, a beautiful greenish refulgence.

Text-figure 6, from Annandale, gives the under side of the head of each of the three definitely established Aëtobatines, *A. narinari*, *A. flagellum*, *A. guttata*. The subjoined table, in which certain characters in these three rays are compared, is also from Annandale. The data for *A. narinari* are taken by Annandale from Jordan and Evermann's text and figures (1896 and 1898), that for the other two from fresh specimens of the Indian forms.

It will be noted that Annandale's description agrees with Lacépède's figure quite well. It is to be regretted that he has not given us a more minute description of this ray, whose very existence has been much doubted, and especially that he has not given us a figure. In his paper he does not indicate that *A. narinari* is found in the Indian Ocean, and possibly if the matter were gone into with sufficient care and accuracy it might be found that all these Indian and Pacific Aëtobatines with spots only on the posterior half of the body belong under *A. guttata*.

Character.	*A. guttata.*	*A. narinari.*	*A. flagellum.*
Snout...........	Conical, bluntly pointed, distinctly retroverted, at least as broad at the base as long.	Rounded at the tip, much broader at the base than long, straight (?).	Pointed, straight, much longer than broad at the base.
Coloration of dorsal surface.	Uniform dark slate gray in the young, ornamented with bluish spots, which are confined to the posterior half of the disk in the adult.	The whole disk including the head covered with whitish spots both in the young and the adult.	Disk in the adult of a uniform dark greenish bronze color, without spots.
Size.............	Diameter of disk in adult at least 125 cm.	Diameter of disk in adult 51 cm.	Diameter of disk in adult 47 cm.
Habitat.........	Tropical parts of the Indian Ocean.	Both sides of the Atlantic; Gulf of Guinea, American coast as far north as Virginia, West Indies.	Red Sea, Bay of Bengal.

Since the above was written, I have received a copy of Annandale's second paper (1910), which very satisfactorily explains the lack of spots on the anterior parts of *A. narinari*. He is quoted in full on page 271.

AËTOBATUS LATIROSTRIS.

August Duméril in 1861 described from the region of the Gaboon River, west Africa, a new Aëtobatine, *A. latirostris*, differing from *A. narinari* in having a shorter and broader snout, with more pronounced cephalic fins, and fewer, larger, and more widely spaced spots; further, the posterior appendages, tail, and ventral fins are much more markedly separated from each other and from the hinder part of the pectorals. His plate is herein reproduced as figure 8, plate IV. Duméril states that 50 spots on the pectorals averaged 8 to 9 mm. in diameter, while 100 spots from the same region in *A. narinari* averaged 4 to 6 mm.

In 1869, Gunther found a spotted ray from the Bay of Panama to be almost identical with the foregoing, differing from it only in that "the soft rostral appendage is naturally turned upwards like the nose-leaf in certain Chiroptera, and is not stretched forward as represented by M. A. Duméril." This may be true of this form, but fresh Key West and Beaufort specimens have the snout almost straight (see figures in plate VI). Specimens in formalin have the rostral parts somewhat distorted, the snout being more tilted than in nature. Specimens kept in a barrel had the snouts badly distorted by pressure against the sides of the barrel. Gunther's preserved specimen had probably suffered in the same way.

In 1870 volume 8 of Gunther's "Catalogue of the Fishes in the British Museum" appeared and in 1880 his "Guide to the Study of Fishes" was published. In neither is there reference to any Aëtobatine other than *narinari*, *A. latirostris* being made a synonym of *A. narinari.*

Whether or not these rather marked differences are weighty enough to constitute this west African form a separate species, the writer is too little versed in taxonomy to say, and when so great an authority as Gunther pronounces adverse judgment, he can but keep silent. It should be noted, however, that Gill (1867) recognized that his *A. laticeps* is very near to *A. latirostris*, and further let it be remembered that Jordan (1895 and 1898) finally rejects *A. laticeps* as not being specifically different from *A. narinari.*

UNESTABLISHED GENERA AND SPECIES.

It will be recalled that in 1816, Blainville enumerated ten species under the genus *Aëtobatus.* Of these *narinari* and *flagellum* seem to be genuine, while *filicaudatus*, *nichhofii*, *ocellatus*, *fosteri*, etc., seem to fall under other genera of the family Myliobatidæ or may possibly be duplicate names of other Aëtobatines.

In 1823, Temminck published a letter from Van Hasselt, in which the latter speaks of establishing a new species, *Myliobatus cyclura*, very similar to Russell's spotted ray. The language is so obscure and the description so imperfect that I have been able to make out only the fact that Van Hasselt thought that he had a ray differing from Russell's ray only in specific characters. This reference seems to be entirely isolated.

Richardson (1846), in his "Ichthyology of China," lists a ray under this heading "?*Myliobatis oculeus.*" His description is taken from an unpublished drawing in Reeves's collection. He is undecided whether this beautifully spotted ray is "a *Myliobatis* or *Aëtobatis* which is perhaps only a variety of *M. maculatus.*" He did not see the fish and hence could say nothing of the jaws, the deciding structures. Gunther (1870), however, makes this name synonymous with *M. milous*.

Gill (1893) gives the name *A. tenuicaudatus* for an Australian form, making it synonymous with Hector's *Myliobatis tenuicaudatus*. Reference to Hector (1877), however, throws it out of the genus *Aëtobatus*, since it has teeth in seven rows in each jaw.

Starks and Morris (1907), in their paper on the "Marine Fishes of Southern California," list an *Aëtobatus californicus*. However, Mr. Starks in a recent letter to the present writer, says that this ray does not have angled teeth and that he now calls it *Myliobatis californicus*.

Last of all, Gunther (1910) reverts to the fact that in 1870 he put all Aëtobatines into one species, *A. narinari*, notes that the characters upon which the species are based consist of differences in the length of the snout and in the distribution of the spots, and concludes by saying that he is not yet persuaded that he was in error in 1870, since forms with snouts intermediate between long and short and forms with and without spots are of frequent occurrence.

However, on turning a page we find this author describing, under the name *Aëtobatis punctata*, a spotted ray from the south Pacific synonymous with Miklouho-Maclay and Macleay's *Myliobatis punctatus* (1886). The plate of these latter writers, giving various views of this fish, is reproduced herein as figure 21, plate VIII. If this is not sufficient to throw this ray out of the genus *Aëtobatus* (see mouth in the figures) then let it be recalled that these writers in their text say "Teeth-plates of many longitudinal rows of teeth" (see their drawing). That it is, however, a transitional or intermediate form would seem to follow from the further statement that the middle rows of teeth are largest and that the teeth-plates of the upper jaw are nearly twice as wide as the lower, the proportion being that of 48 to 27.

Snyder, in his "The Fishes of Okinawa, One of the Riu Kiu Islands" (1912), lists a species, *Aëtobatis tobijei*, new to the present writer, but gives no data whatever about it. But Jordan and Fowler (1903), in their "Review of the Elasmobranchiate Fishes of Japan," call this same ray *Myliobatis tobijei* and note that its teeth are pavement-like in several series in each jaw. Furthermore Bleeker (1854), the original describer of this ray, names it *M. tobijei* and says "Upper jaw broader than long with laminated teeth; lower longer than wide, with median hexagonal teeth varying from more than twice to more than three times broader than wide." From this we see that Snyder's fish is not an *Aëtobatus* at all, but a *Myliobatis*.

SYNONYMY.

Synonymy of the Spotted Eagle Ray, Aëtobatus narinari, in Chronological Order.

1614. *Narinnary.* Abbeville, Hist. Mission Pères Capucins Isle Maragnan, p. 245, Brazil.

1633. *Narrinnari.* Laet, Novus Orbis, Seu Descript. Indiæ Occid., p. 616, Brazil.

——. *Narinari.* Johann Moritz von Nassau Siegen, Brazilianisch Naturgegenstände, I, Tafel 332, Brazil.

——. *Narinari.* Theatri Rerum Naturalium Brasiliæ, Tome I, Icones Aquatilium, fig. 31, Brazil.

1648. *Narinari.* Marcgrave, Historia Rerum Naturalium Brasiliæ, pp. 175–176, figure, Brazil.

1648. *Narinari.* Piso, De Medicina Brasiliensi, p. 44, Brazil.

1658. *Narinari.* Piso, Historia Naturalis & Medica Indiæ Occidentalis, pp. 58 and 293, figures, Brazil.

1686. *Narinari.* Willughby, De Historia Piscium, p. 66, tab. C, fig. 5.

1697. *Pastinaca marina.* Sloane, Phil. Trans., vol. XIX, pp. 674–676, figure, teeth, Jamaica.

1713. *Narinari.* Ray, Synopsis Methodica Avium & Piscium, p. 24.

1718. *Narinari.* Ruysch, Theatrum Universale Omnium Animalium, Piscium, etc., p. 146, tab. XXXIX, fig. 6.

1725. *Whip Ray.* Sloane, Natural History of Jamaica, pp. 276–277, Jamaica.

1756. *Whip Ray.* Browne, Civil and Natural History of Jamaica, p. 459, Jamaica.

1767. *Narinari.* Jonston, Historia Naturalis de Piscibus et Cetis, pp. 208–209, tab. XXXIX, fig. 6.

1790. *Raja narinari.* Euphrasen, Kong. Svens. Vet. Nya Handl., XI, pp. 217–219, tab. X. First binomial name, species established. St. Bartholomew, West Indies.

1792. *Raja narinari.* Walbaum, Artedi's Bibliotheca Ichthyologica, III, p. 528.

1801. *Raja narinari.* Bloch and Schneider, Systema Ichthyologiæ, p. 361.

1803. *Raja ocellata.* Russell, Fishes of Coromandel, p. 5, pl. VIII, Coromandel.

1816. *Aëtobatus narinari.* Blainville, Journal de Phys., de Chem., et d'Hist. Nat., LXXXIII, pp. 261–262. Generic name first established.

1817. *Raja narinari.* Cuvier, Le Regne Animal, t. II, p. 138.

1830. *Myliobatis narinari.* Bennett, Memoir Life of Raffles, p. 694. East Indies.

1835. *Myliobatis eeltenkee.* Ruppell, Fische des Rothen Meeres, Neue Wirbelthiere, Fauna Abyssinien, pp. 70–71, fig. teeth, Red Sea.

1836. { *Raja narinari.* / *Aëtobatis narinari.* / *Myliobatis narinari.* } Agassiz, Poissons Fossiles, III, pp. 79–80 and 325, tab. D, figs. 1 and 2; tab. 46, figs. 4 and 5 (all teeth).

1839. *Aëtobatis indica.* Swainson, Nat. Hist. Fish. II, p. 321.

1841. *Aëtobatis narinari.* Müller and Henle, Systematische Beschreibung Plagiostomen, pp. 179–181. The form first definitely established under full diagnosis of characters.

1817. *Myliobatis narinari.* Cuvier, Le Regne Animal, vol. Poissons, pl. 118, No. 4*a* (teeth).

1844. *Raja edentula.* Forster (Lichtenstein ed.) Descriptiones Animalium, etc., pp. 227–229, South Seas.

1840 to 1845 } *Aëtobatis narinari.* Owen, Odontography, pp. 46–48, pl. 16, fig. 1 (section through dried head).

1849. *Sloasodon narinari.* Cantor, Jour. Asiatic Soc., Bengal, XVIII, pp. 434–435, Bay Bengal.

1852. *Aëtobatis narinari.* Bleeker, Verh. Bataav. Gen. Kunst. en Weten., XXIV, p. 88, East Indies.

1859. *Goniobatis meleagris.* Agassiz, Proc. Bost. Soc. Nat. Hist., VI, p. 385, Sandwich Islands.

1861. *Aëtobatis narinari.* Duméril, Arch. Mus. Hist. Nat., x, pp. 241–243, pl. 20, fig. 2 (head).
1865. *Aëtobatis narinari.* Duméril, Hist. Nat. Poissons, i, p. 640.
1865. *Aëtobatis narinari.* Day, Fishes of Malabar, pp. 280–281, Indian Ocean.
1867. *Aëtobatis laticeps.* Gill, Ann. Lyc. Nat. Hist. New York, viii, p. 137.
1870. *Aëtobatis narinari.* Günther, Cat. Fishes Brit. Mus., viii, pp. 492–493.
1871. *Aëtobatis narinari.* Klunzinger, Verh. K. K. Zool-Botan. Gesell. Wien, xxi, pp. 685–686, Red Sea.
1877. *Aëtobatis narinari.* Yarrow, Proc. Acad. Nat. Sci. Phila., xxix, p. 216. Beaufort.
1878. *Aëtobatis narinari.* Day, Fishes of India, ii, p. 743, fig. 4, pl. cxciv. Indian Ocean.
1879. *Aëtobatis narinari.* Jordan and Gilbert, Proc. U. S. Nat. Mus., i, p. 386, Beaufort.
1880. *Aëtobatis narinari.* Günther, Introd. Study Fishes, pp. 344–345, fig. 130.
1881. *Aëtobatus narinari.* Poey, Fauna Puerto-Riqueña, p. 349.
1882. *Aëtobatis narinari.* Jordan and Gilbert, Synopsis Fishes North America, p. 50.
1883. *Aëtobatus narinari.* Stahl, Fauna de Puerto Rico, pp. 81 and 167.
1886. *Aëtobatis narinari.* Douglas-Ogilby, Proc. Linn. Soc., New South Wales, x, p. 466, New South Wales.
1886. *Aëtobatis narinari.* Günther, Encyc. Britan., xx, p. 300, fig. 3.
1887. *Stoasodon narinari.* Jordan, Proc. U. S. Nat. Mus., ix for 1886, pp. 26 and 558, Beaufort and West Indies.
1895. *Stoasodon narinari.* Henshall, Bull. U. S. Fish Comm., xiv, p. 210, W. Coast Florida,
1895. *Aëtobatus narinari.* Jordan, Proc. Calif. Acad. Sci., ser. 2, v, p. 381, West Coast of Mexico.
1896} *Aëtobatus narinari.* Jordan and Evermann, Fishes North and Middle America.
1900} i, 88; iv, pls. xv and xvi, figs. 37 and 37*a*.
1896} *Aëtobatus laticeps.* Jordan and Evermann, Fishes North and Middle America, i,
1898} p. 88–89; iii, p. 2753.
1900. *Aëtobatus narinari.* Evermann and Marsh, Bull. U. S. Fish Comm., xx, pt. i, p. 67. Porto Rico.
1901. *Aëtobatis narinari.* Steindachner, Denk. Kais. Akad. Wiss., Wien, lxx, p. 517, Laysan.
1904. *Aëtobatus narinari.* Jenkins, Bull. U. S. Fish Comm., xxii, p. 421, Honolulu.
1905. *Stoasodon narinari.* Jordan and Evermann, Bull. U. S. Fish Comm., xxiii, pt. i, pp. 49–50, Hawaii.
1905. *Stoasodon narinari.* Jordan and Thompson, Bull. U. S. Bureau Fisheries, xxiv, p. 232, Tortugas.
1905. *Aëtobatus narinari.* Jordan, Guide to Study of Fishes, i, p. 557.
1907. *Aëtobatus narinari.* Smith, Fishes of North Carolina, p. 46, Beaufort.
1907. *Stoasodon narinari.* Jordan and Seale, Bull. U. S. Bureau Fisheries, xxvi, p. 4, Cavite, P. I.
1907. *Aëtobatus narinari.* Miranda Ribeiro, Arch. Mus. Nac. Rio Janeiro, xiv, Coast of Brazil.
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1910. *Aëtobatus narinari.* Gudger, Am. Nat., xliv, pp. 399–400, Beaufort.
1910. *Aëtobatis narinari.* Annandale, Mem. Ind. Mus., pp. 4–5, pl. ii, fig. 2 (young), Bay of Bengal.
1912. *Aëtobatus narinari.* Gudger, Proc. Biol. Soc. Wash., xxv, pp. 150–152.
1913. *Aëtobatus narinari.* Gudger, Proc. Biol. Soc., Wash., xxvi, p. 101.
1913. *Aëtobatus narinari.* Coles, Bull. Am. Mus. Nat. Hist., xxxii, pp. 30–33, figs. 1–2; pl. iii, figs. 1, 2, 3.

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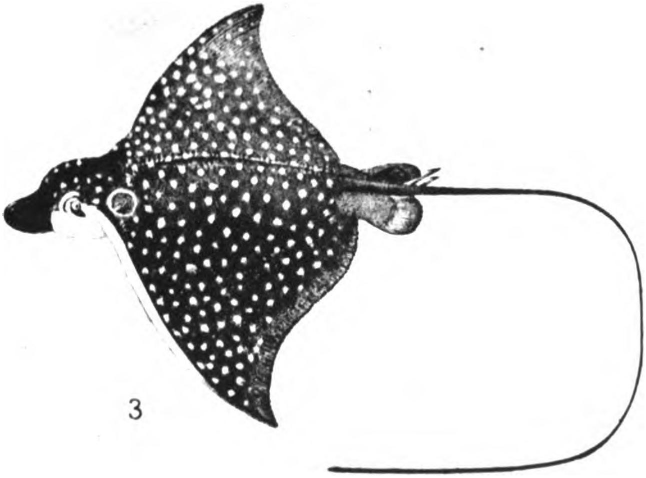

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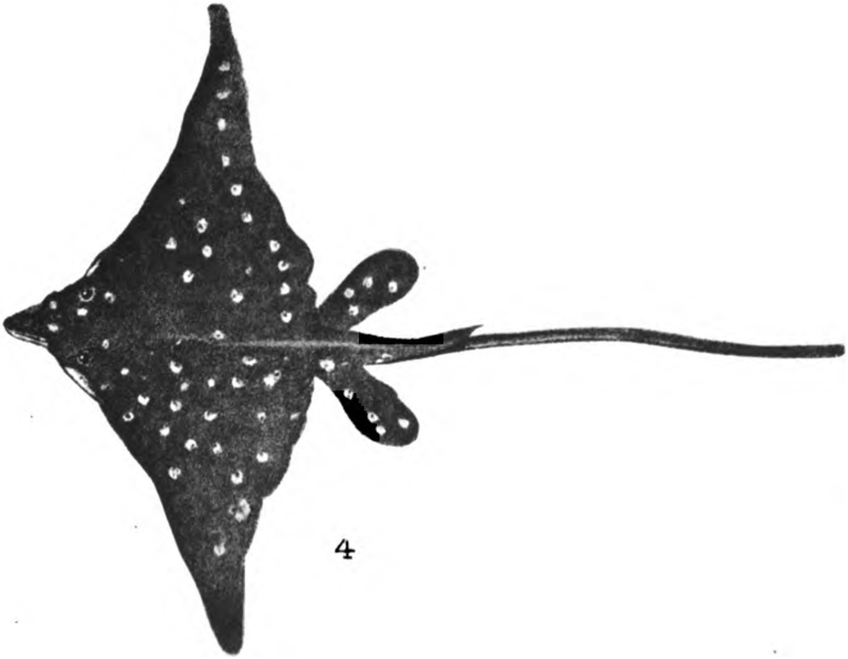

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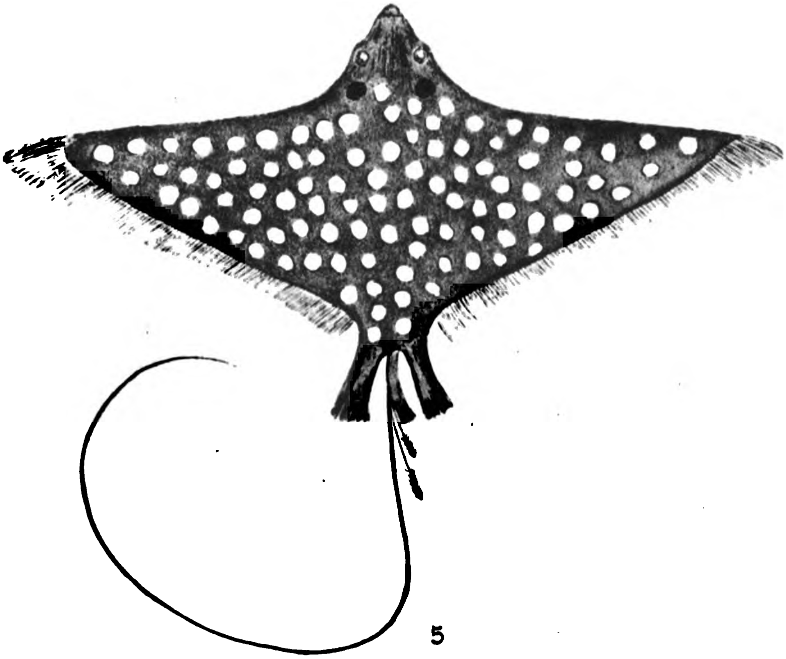
FIG. 3. *Narinari*, after Marcgrave's water-color painting in the Royal Library of Berlin. Brazil, 1648.

FIG. 4. *Narinari*, after Poste's oil painting in the Royal Library of Berlin. Brazil 1648.



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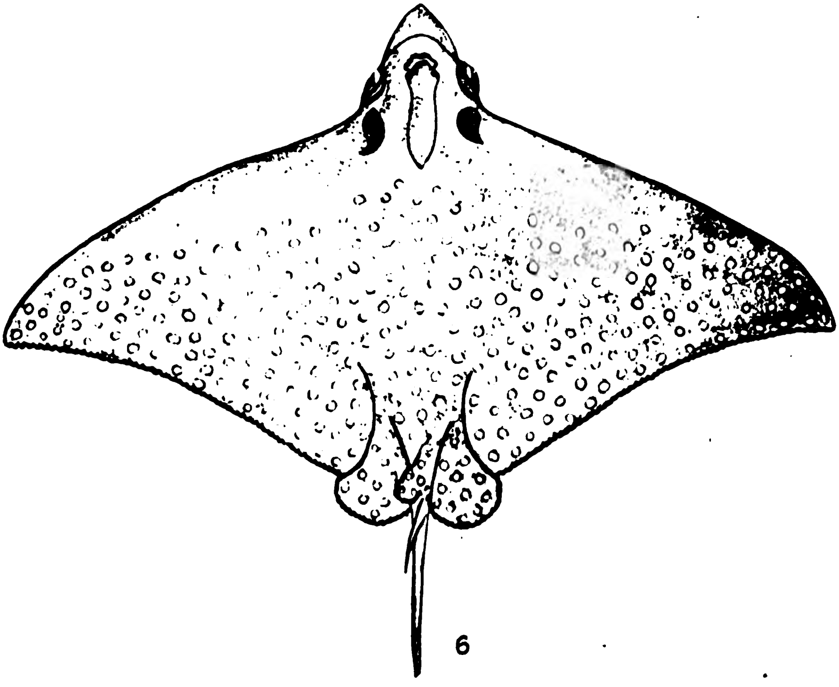

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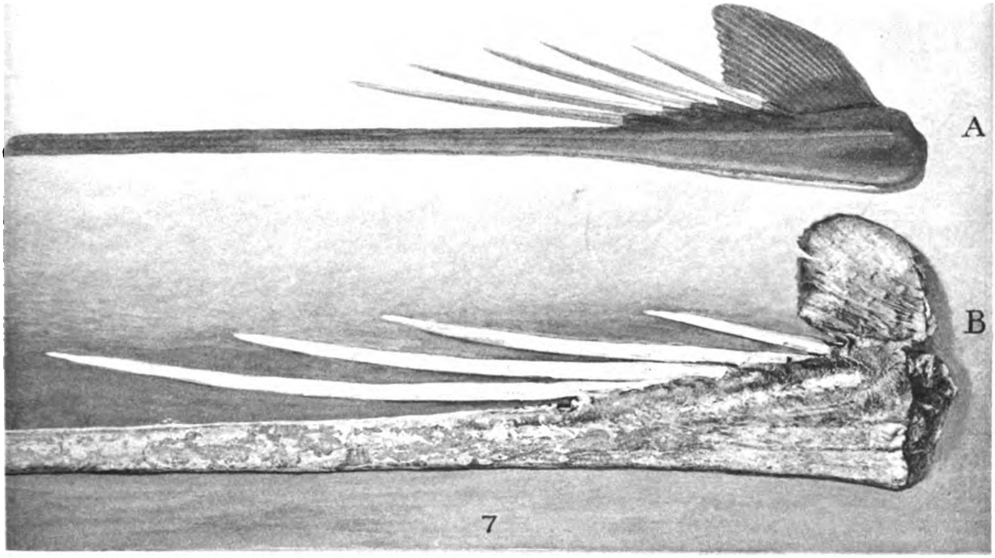
FIG. 5. *Raja narinari*, after Euphrasen, 1790, West Indies.
FIG. 6. *Raja ocellata*, after Russell, 1803, Coromandel.



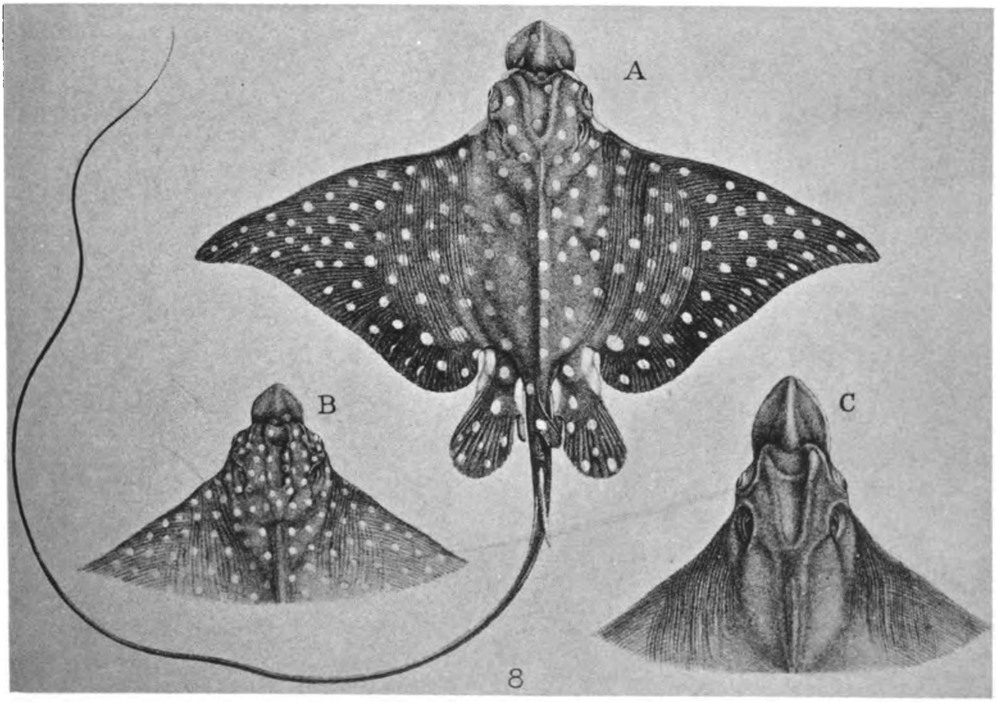
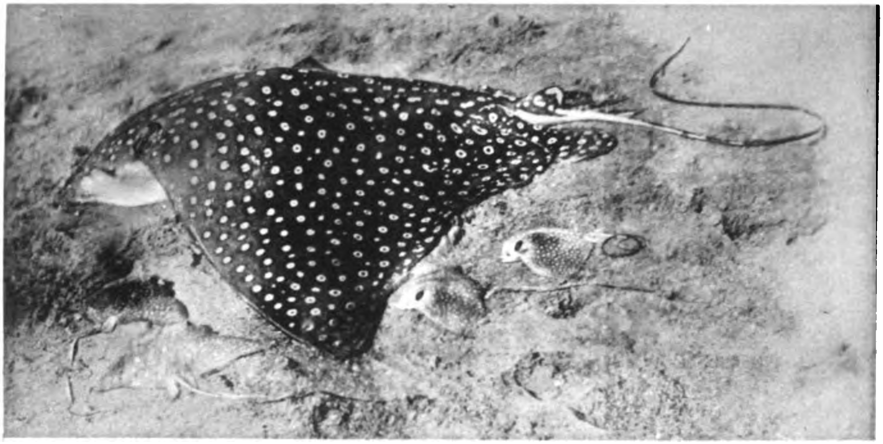


FIG. 7. A. Tail of *Raja quinqueaculata*, after Quoy and Gaimard, 1824, Guam. B. Tail of *A. narinari*, Beaufort.

FIG. 8. A. *A. latirostris;* B. *A. narinari;* C. *A. fouet* (*A. flagellum?*) after A. Dumiril, 1865.



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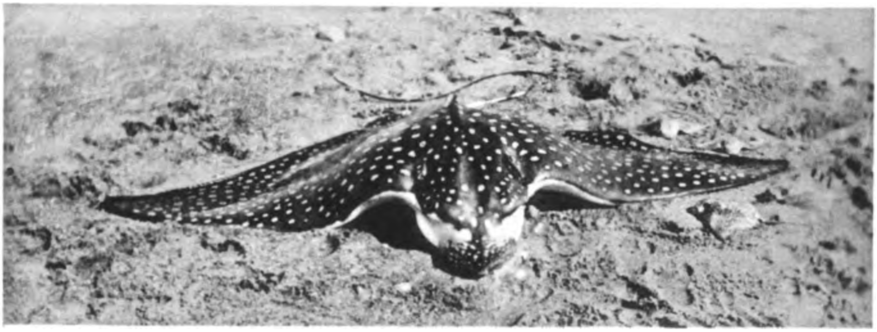

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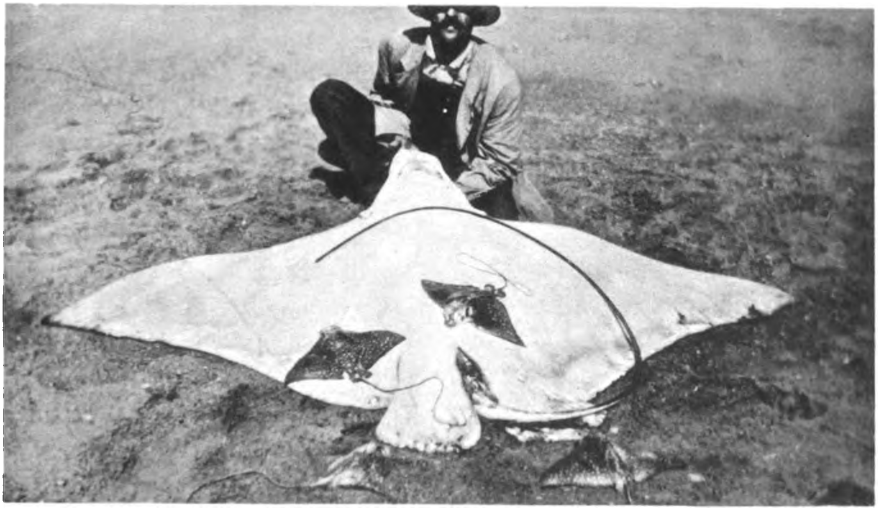

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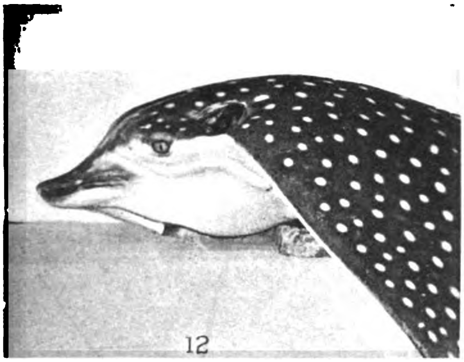
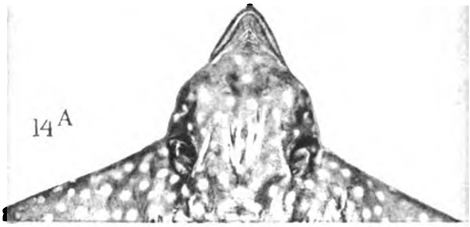
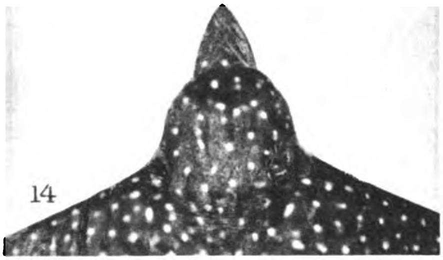
Aetobatus narinari and Embryos. After Coles 1913.

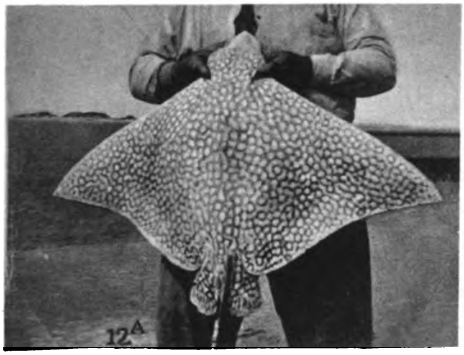
A. narinari (Euphrasen); female, 7 ft. 2 in. in diameter, and the four embryos to which she gave birth at intervals of a few seconds, on the beach. Cape Lookout, N. Carolina.

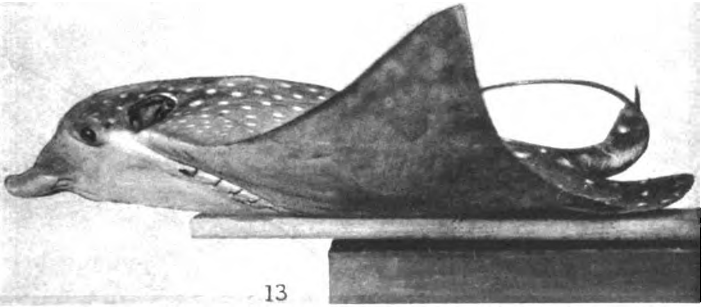
FIG. 9. Seen from the side. FIG. 10. From in front. FIG. 11. From below. In figure 9 note unusual coloration consisting of ocelli instead of white spots.

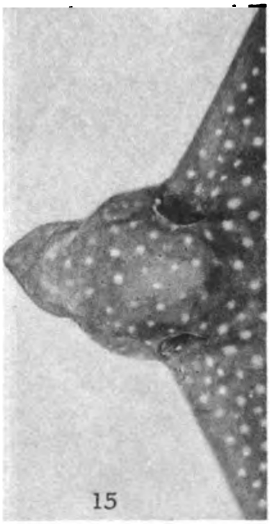












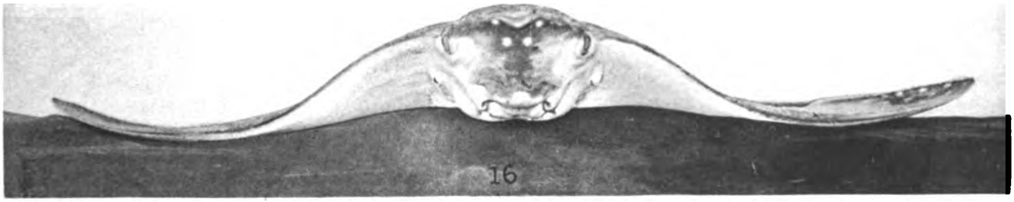
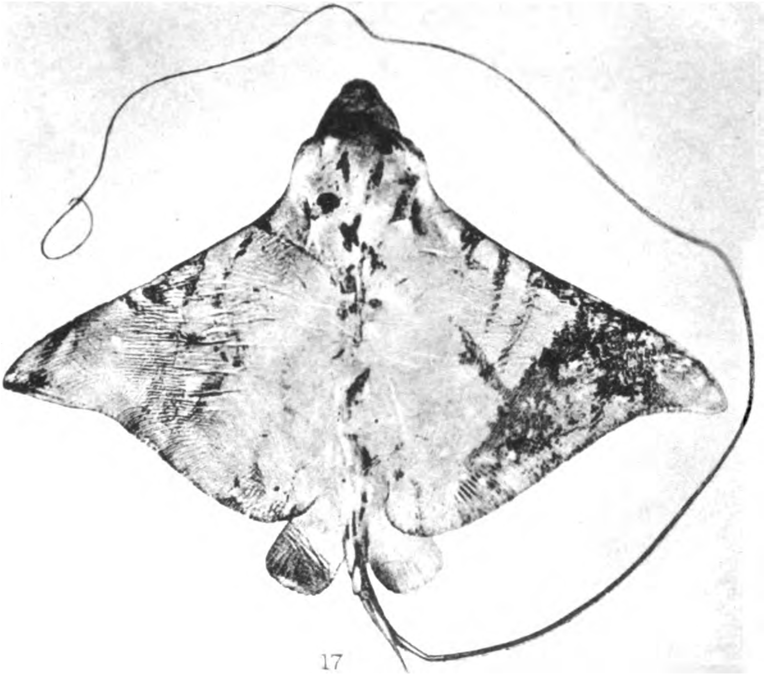


FIG. 12. Lateral view of head of *A. narinari*, Beaufort, 1910 (fresh specimen).
12A. *A. narinari*, an albino female taken at Cape Lookout, 1913.
FIG. 13. Lateral view of *A. narinari*, Beaufort, 1909 (formalin specimen).
FIG. 14 and 14A. Heads of *A. narinari*, Beaufort, 1910 (fresh specimen).
FIG. 15. Head of specimen shown in figure 13.
FIG. 16. Front view of Spotted Eagle Ray, Beaufort, 1910 (fresh specimen)



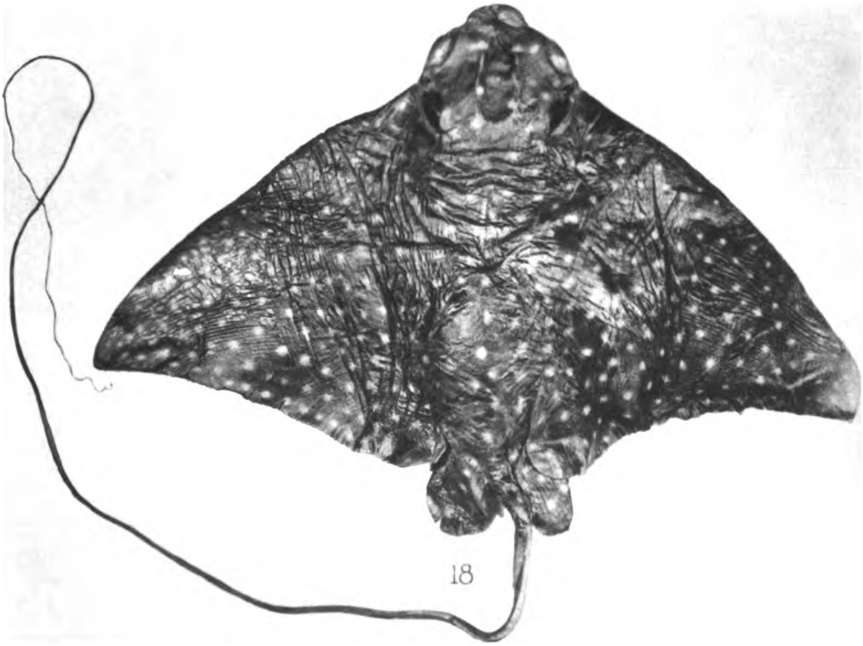
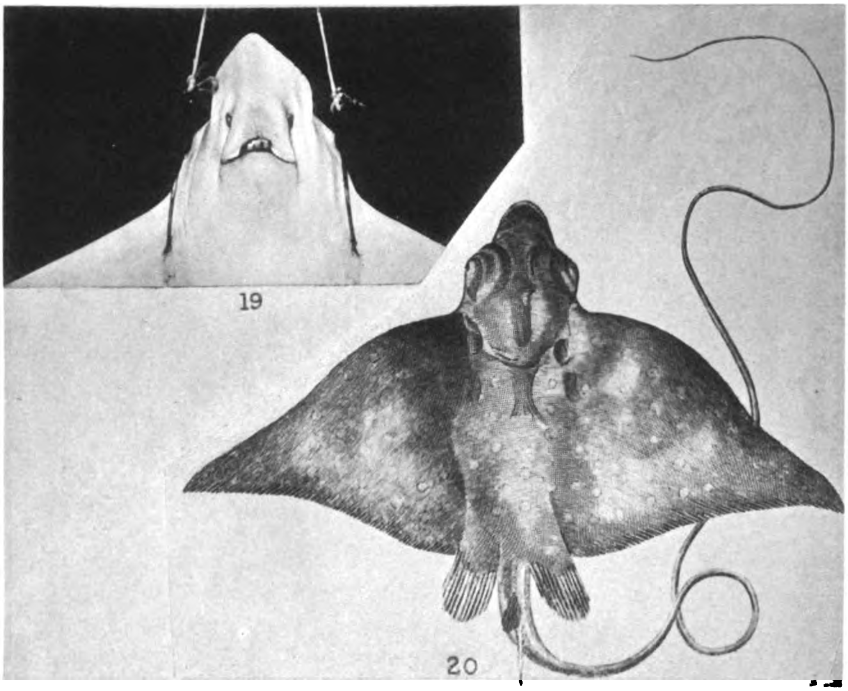


Fig. 17. *A. narinari*, young, after Annandale, 1910, Bay of Bengal

Fig. 18. Embryo of Spotted Eagle Ray. One of the four obtained from the ray shown in Plate V.



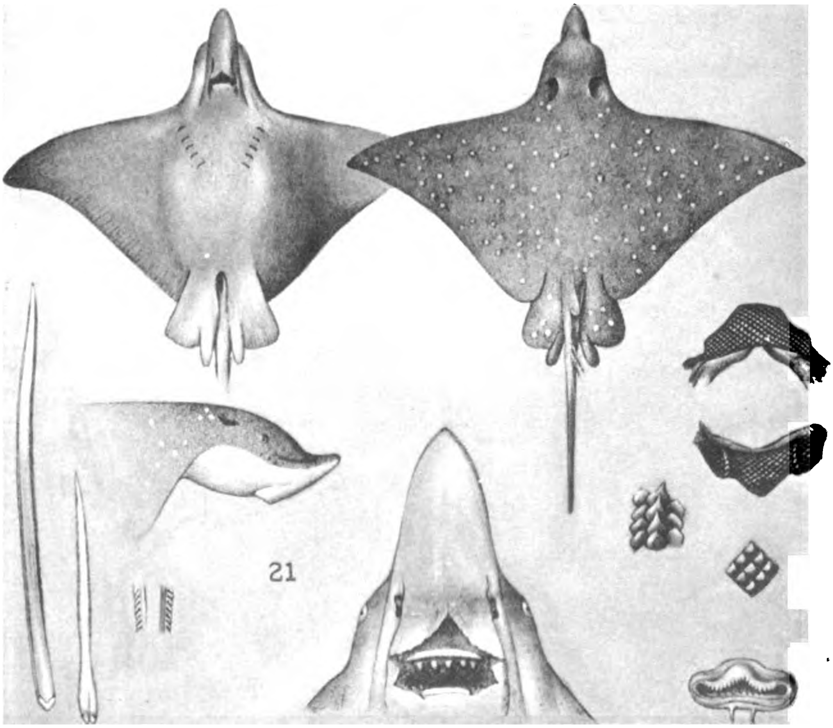
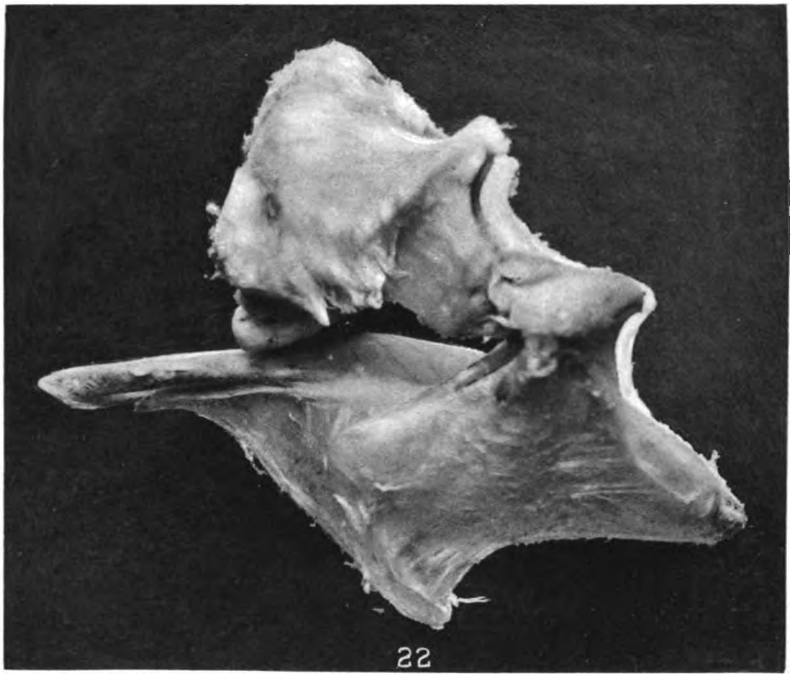


Fig. 19. Ventral view of head of ray shown in figures 13 and 15, plate VI.

Fig. 20. *A. guttata*, after Shaw-Lacepede-Commerson.

Fig. 21. *Myliobatis punctatus*, after Miklouho-Maclay and Macleay, 1886, Hermit Islands.



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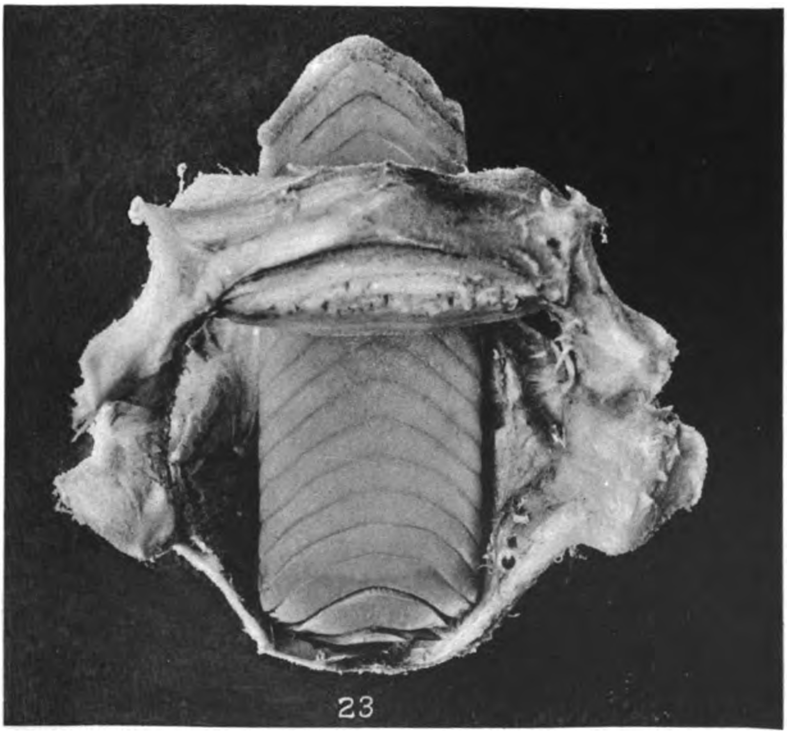

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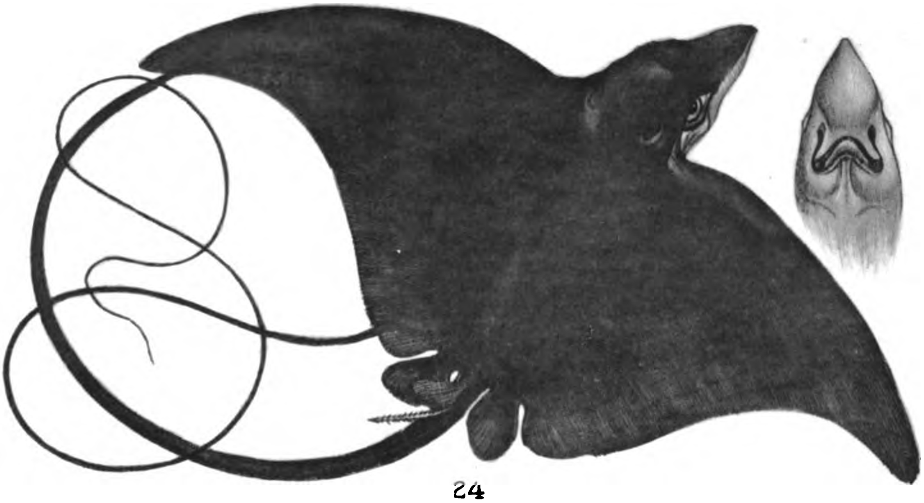
FIG. 22. Side view of jaws of *A. narinari* from Beaufort.
FIG. 23. Jaws of *A. narinari* seen from above.



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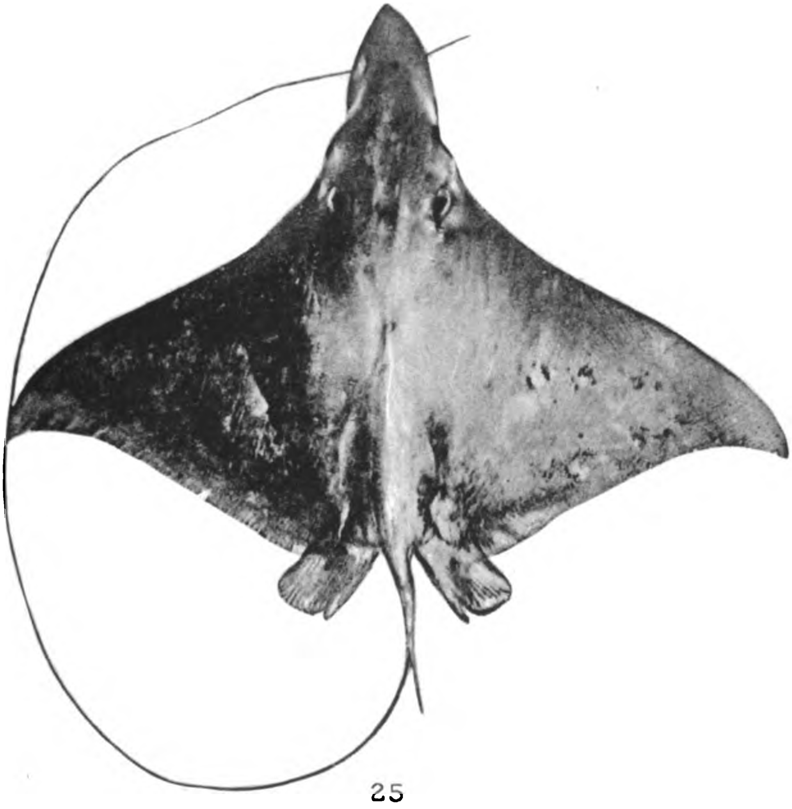

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Fig. 24. *Raja flagellum*, after Bloch and Schneider, 1801.
Fig. 25. *Aetobatus flagellum*, after Annandale, 1910, Bay of Bengal.

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